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EFFECTS OF INTRAVENOUS INJECTIONS OF THE ETHER-INSOLUBLE FRACTION OF LIPOIDS OF BEEF BRAIN

A COMPARISON WITH THE LIPOID STORAGE DISEASES AND WITH THE EFFECTS OF INJECTIONS OF PHOSPHATIDES ALONE

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It has been shown that phospholipids call forth macrophages and are quickly absorbed without residual reaction when they are injected subcutaneously, while galactolipids elicit chiefly neutrophils and fibrous tissue and are poorly absorbed.[1] When mixed with phospholipids, however, the galactolipids are absorbed and materially modify the character of the macrophages.[2] On the other hand, both the phospholipid sphingomyelin [3] and the galactolipid kerasin [4] are stored in macrophages throughout the organism pathologically, in association with variable amounts of glycerophosphatides,[5] while the glycerophosphatides themselves have not been specifically implicated in a storage disease. Sphingomyelin and kerasin have a common radical, the sphingosine-lignoceric bond, and Epstein [5] suggested that pathologic storage of either lipoid may be due to disturbance of the metabolism of phosphoric acid alone, of such nature that hyperactivity results in accelerated linkage of the phosphoric acid radical with the sphingosine-lignoceric one and consequent overproduction of sphingomyelin, while hypoactivity results in decreased linkage, with liberation of the sphingosine-lignoceric radical for union with galactose and consequent overproduction of kerasin. Epstein [5] and Kimmelstiel and Laas [6] expressed the belief that

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1. Tompkins, E. H.: Bull. Johns Hopkins Hosp. **70**:55, 1942.
2. Tompkins, E. H.: South. M. J. **33**:154, 1940.
3. Klenk, E.: Ztschr. f. physiol. Chem. **229**:151, 1934.
4. Lieb, H.: Ztschr. f. physiol. Chem. **140**:305, 1924.
5. Epstein, E.: Ergebn. d. allg. Path. u. Path. Anat. **33**:280, 1937.
6. Kimmelstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**:417, 1934.

galactolipids can enter cells only in the presence of phospholipids. In view of these concepts, therefore, and the specificity of the reactions of the subcutaneous tissues to individual lipoids, it seemed important to determine the systemic effects from intravenous injections of the same lipoids when given individually or in known admixture with other lipoids.

The effects of intravenous injections of the glycerophosphatide lecithin have been reported.[7] The preparation of the sphingomyelins and galactolipids in sufficient quantities for repeated intravenous administration to enough animals to form representative series offered considerable difficulty. Therefore the experimental procedure was adopted of injecting into a large series of animals the biologic mixture of both substances which is present in the ether-insoluble fraction of the lipoids of beef brain and comparing the results with those obtained by injecting similarly into a smaller series of animals the purified sphingomyelins or galactolipids extracted from the mixture. Ferraro and Jervis [8] recently reported the pathologic changes in rabbits following repeated intravenous injections of sphingomyelin alone. The present data represent the findings obtained from intravenous injections of the mixture of sphingomyelins and galactolipids present in beef brain. They include certain aspects not investigated by Ferraro and Jervis and, in addition, serve as a basis of comparison with the effects from injections of either group of phospholipids alone, i. e., sphingomyelin and the glycerophosphatide lecithin.

METHODS

The material used for these experiments was purified from a dry benzene extract of beef brains which was donated by Dr. David Klein, Wilson Laboratories, Chicago.

Two different lots of material were prepared from this. The first was employed for the mice used in these studies and for the first 2 rabbits; the second was used for the remaining rabbits (3 to 13 inclusive). The crude benzene extract was dissolved in large amounts of warm ethyl ether. The solutions were allowed to stand in tall containers at 40 C. until clear. The supernatant fluids were decanted; the sediments were redissolved in warm ether, and the procedure was repeated until the supernatant fluids were practically colorless. The final sediments were stirred in several changes of warm acetone and allowed to dry in air. They were ground to a fine powder before being weighed and were made into 1 per cent stock solutions in equal parts of chloroform and ethyl alcohol. The solutions were filtered through fine paper several times in order to remove insoluble matter which had been carried along with the ether-insoluble residues. The amount of this differed with each lot. The percentages of the filtered solutions were obviously reduced accordingly.

7. Tompkins, E. H.: Arch. Path. **35**:695, 1943.

8. Ferraro, A., and Jervis, G. A.: Arch. Path. **30**:731, 1940.

Emulsions for injection were prepared fresh each day. The desired amounts of the stock solution were measured into beakers, evaporated over a water bath, made into 5 per cent emulsions in 5 per cent dextrose solution and sterilized for two hours in a steam sterilizer.

The injections were given at the rate of one every twenty-four hours, six days a week, in the aural veins of nonpregnant adult female rabbits. The daily doses were increased gradually from 0.01 Gm. to constant levels varying from 0.05 to 0.70 Gm. (i. e., 1 to 14 cc.). The injections were given slowly over a period of three or more minutes. Under these conditions they were well tolerated with one exception (rabbit 6), both at the time of injection and throughout the experimental course. The rabbits were always quiet for several hours after receiving an injection but exhibited signs of respiratory distress only occasionally. A more con-

TABLE 1.—*Details of the Experiments*

Rabbit	Duration of Course of Injections, Days	Dosage			Weight of Rabbit, Gm.*	Hemopoietic Organs *			
		Total Amount Given, Gm.	Maximum Daily Dose			Spleen, Gm.	Liver, Gm.	Marrow	
			Amount, Gm.	Number of Days Given				Estimated Activity	Myeloid-Erythroid Ratio
1	63	2.4	0.05	46	3,530	Firm	Normal	+++	0.9
2	63	6.5	0.20	10	3,480	Firm	Normal	+++	0.8
3	36	7.5	0.35	16	2,340	Large	Large	..	...
11	20	2.8	0.25	3	2,680	Large	Large	++	...
6	51	13.9	0.50	8	2,700	3 × normal	2 × normal	+++	...
5	55	16.2	0.50	12	3,220	2 × normal	Large	++	1.7
10	57	17.1	0.50	13	3,550	3 × normal	2 × normal	++	2.7
4	59	17.2	0.50	15	3,330	11.5	150.4	+++	1.3
8	71	23.6	0.70	3	3,480	7.2	150.5	+++	3.8
13	73	23.9	0.70	4	3,400	9.8	104.5	+++	1.2
9	73	24.9	0.70	5	2,650	11.6	87.9	+++	3.5

* The averages of corresponding weights from 9 normal control rabbits killed in the same manner as the experimental animals are as follows: rabbit, 3,800 Gm. (2,980-4,500); spleen, 2.4 Gm. (1.13-3.72); liver, 94.6 Gm. (68-110); marrow, estimated activity ++; myeloid-erythroid ratio, 1.34 (0.8-2.4).

centrated dose, on the other hand, proved immediately fatal to a rabbit [11] which had apparently tolerated the lower concentration without discomfort, and a dose of standard concentration but of a size usually attained only progressively also proved immediately fatal to a rabbit which had not been habituated to the experimental material. The injections were continued over intervals of time which varied from twenty to seventy-three days. The data concerning the material injected, the dosages and the duration of the course of injections are presented in table 1.

The rabbits were kept, fed, exercised and clipped exactly in the manner described for the rabbits given lecithin.[7] They remained in excellent condition, held or gained weight and were normally active. The fur and corneas remained healthy.

Total white and differential (supravital technic) blood cell counts were made several times a week throughout the experimental course. The blood was always drawn by puncture of an aural vein shortly before the time for an injection. Trenner automatic pipets and Levy-Hausser counting chambers were used. The

technic employed was that described for the rabbits given lecithin.[7] Total red cell counts were made infrequently. All statements of counts are based on absolute numbers.

Serial counts were also made throughout twenty-four hour periods following individual injections of the series. They revealed characteristic curves and presented problems which are essentially distinct from the general systemic effects. These were investigated in further detail therefore and have been presented in abstract[9a]; they are to be reported separately.[9b]

The animals were put to death by being given illuminating gas or ether or by intravenous injection of soluble pentobarbital or air in lethal amounts. As soon as respiration ceased, the abdomen was opened, the inferior vena cava cut and the blood allowed to drain, with the animal held upright. The organs were then examined grossly, and blocks were saved in Helly's or Bouin's fixatives. Blocks of the marrow were saved in Kingsley's fixative.[10] Scrapings of the hemopoietic organs were examined supravitally. All tissues were routinely embedded in paraffin and cut at 3 microns, and the sections were stained with hematoxylin and eosin. The sections of marrow and those of spleen fixed in Helly's fluid were also stained with Kingsley's stain.[11] The sections of marrow were counted differentially. The prussian blue test for iron (Key's technic) was applied to sections of the hemopoietic organs both before and after treatment with nitric acid or trypsin for masked iron.

Twelve mice were also given injections of the lot of material used for rabbits 1 and 2. One to 5 per cent emulsions were given every two to three days in a vein of the tail, in amounts of 0.005 to 0.010 Gm. The experimental periods extended from four to sixty-four days. The tissues were studied only after fixation in Bouin's fluid.

EXPERIMENTAL DATA

Studies of Blood.—Figures 1 and 2 (rabbits 4 and 13) illustrate the reactions of the white blood cells in general. The total white cell counts began to increase ten to twenty-three days after the start of the injections (daily doses, 0.05 to 0.35 Gm.). They varied considerably from day to day but were continuously elevated throughout the remainder of the experimental period. In all instances the increase was due to an increment of neutrophils (i. e., pseudoeosinophils) and lymphocytes and in many instances to an increment of monocytes and basophils. The neutrophils began to increase sixteen to forty-six days after the start of the injections (daily doses, 0.15 to 0.50 Gm.); the lymphocytes began to increase five to forty-two days after the start of the injections (daily doses, 0.15 to 0.45 Gm.). The increase varied considerably from animal to animal and was irrespective of dosage. In some instances the counts attained levels that were double or treble the control counts. The monocytes increased in 5 animals, beginning sixteen to fifty days after the start of the injections (daily doses, 0.05 to 0.50 Gm.); they exhibited little change in the other 6 animals. The characteristic macrophages which will be shown to have infiltrated most tissues were never observed in the blood smears.

As has been stated, serial counts over twenty-four hour periods following individual injections revealed characteristic curves,[9a] which were found to be superimposed on the basic levels which the blood had attained at the time of the

9. Tompkins, E. H.: (*a*) Anat. Rec. **79**:59, 1941; (*b*) Bull. Johns Hopkins Hosp., to be published.

10. Kingsley, D. M.: Folia haemat. **57**:87, 1937.

11. Kingsley, D. M.: Stain Technol. **10**:127, 1935.

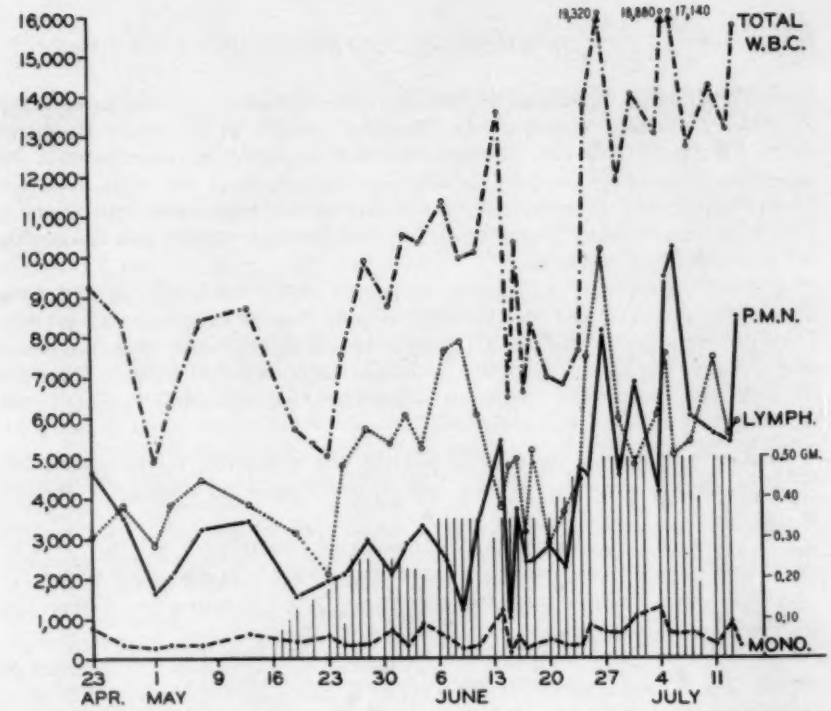

Fig. 1 (rabbit 4).—Curves of total and differential (absolute numbers) white blood cell counts correlated with the daily injections of experimental material.

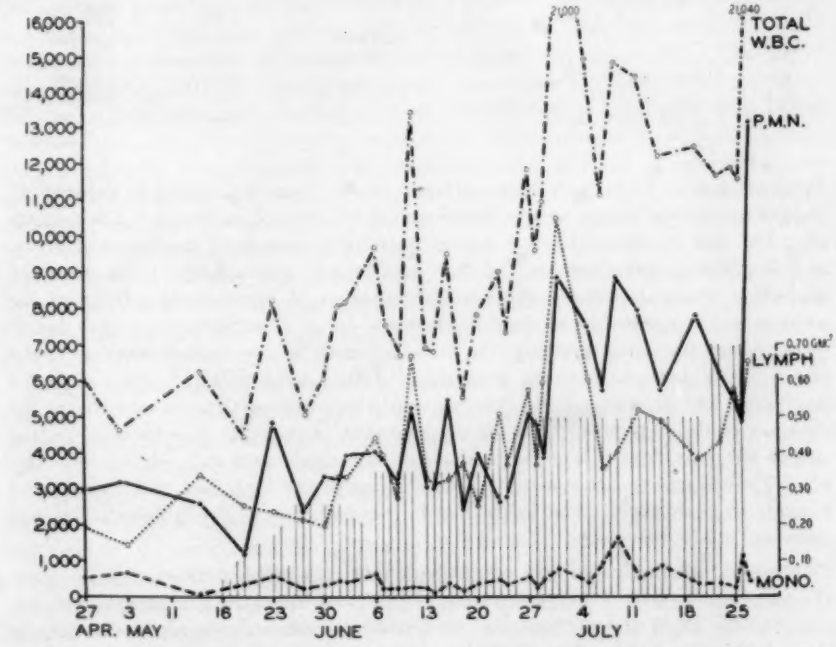

Fig. 2 (rabbit 13).—Curves of total and differential (absolute numbers) white blood cell counts correlated with the daily injections of experimental material.

particular injection. Undoubtedly, these periodic fluctuations incident to individual injections profoundly influenced the sustained changes in the white blood cell counts described. This fact, however, does not invalidate the statement that the experimental injections produced definite sustained effects on the white blood cell counts, which were characterized by increases of the total white cell counts in association with increases of neutrophils and lymphocytes invariably and of basophils and monocytes less regularly.

Red cell counts were made at approximately thirty and sixty days after the start of the injections and are presented in table 2 as of those intervals of time. With the exception of rabbit 1, which received the smallest daily dose, the counts at the end of two months were all moderately subnormal for rabbits. Nucleated red cells were observed in circulation occasionally, but qualitative changes in the erythrocytes were not encountered.

Studies of Marrow.—The femoral marrow was remarkable for its volume and firmness. It was deep rose in color and so firm that it could easily be handled

TABLE 2.—*Red Blood Cell Counts*

	30 Days from Start of Injections		60 Days from Start of Injections	
Rabbit	Daily Dose at Time of Count, Gm.	Red Cell Count	Daily Dose at Time of Count, Gm.	Red Cell Count
1	0.05	5,410,000	0.05	5,000,000
2	0.05	5,350,000	0.20	4,440,000
3	0.35	4,355,000	....	.....
11	....	.....	....	.....
6	0.45	5,290,000	....	.....
5	....	.....	0.50	4,820,000
10	0.35	5,210,000	0.50	4,420,000
4	0.35	4,660,000	0.50	4,180,000
8	....	.....	0.50	3,900,000
13	....	.....	0.50	4,330,000
9	....	.....	0.50	3,887,000

without trauma. Grossly, it appeared very similar from experiment to experiment, irrespective of the dosage or the duration of the course of injections. The sections (fig. 3, *1* and *2*) revealed active hemopoiesis with consequent displacement of fat and, in addition, great numbers of the characteristic macrophages to be described presently. Table 1 presents the estimated degrees of hemopoietic activity of the sections and the ratios of myeloid to erythroid cells. The hyperplasia was due to increased production of granulocytes, as is indicated by the marked increase in the ratios. It was represented by generalized diffuse hyperactivity, which extended throughout the diameter of the marrow and which seemed dependent more on the duration of the experiment and the total amount of material injected than on the size of the daily dose. The cells were mostly late myelocytes and polymorphonuclears. These findings are obviously consistent with the sustained leukocytosis and increase in neutrophils found in the blood. No reflection of the lymphocytosis was observed in the marrows.

General Pathologic Changes.—Pathologic data concerning the hemopoietic organs are presented in table 1. Other than the evidences of increased granulocytic activity found in the blood and the marrow, the pathologic alterations were woven largely about infiltrations of huge macrophages. These were most prominent in the spleen and the lungs but were abundant in all of the hemopoietic organs and occurred

sporadically in all of the other organs. Morphologically, these macrophages were identical in all sites except the lungs, and were the same as the macrophages which had been obtained following subcutaneous injections of mixtures of the same lipoids.[12]

In the supravital preparations the cells varied in diameter from about 25 to 70 microns and the vacuoles from about 2 to 6 microns. The smaller cells contained only stained vacuoles, which were uniformly the shade of neutral red dye in faintly acid solutions. The largest cells usually contained entirely unstained vacuoles of the same general size as those in the stained cells but pale gray. Cells intermediate between these extremes contained mixtures of stained and unstained vacuoles, the latter fading from the deep color of those in the smaller cells to the colorless ones in the larger cells. The vacuoles were usually round to cuboidal or hexagonal in shape, rather chalky in appearance and of low refractivity. It was only rarely that highly refractive droplets of neutral fat were present among these characteristic vacuoles. The cells were rarely observed with other contents, even in the splenic scrapings. The vacuoles stained lilac with nile blue sulfate and with methylene blue and faint brownish gray with osmic acid gas, and did not stain with janus green until cytologic death was under way. Small, fine mitochondria scattered between the vacuoles stained normally with janus green. The vacuoles were not dissolved by acetone and only partly by chloroform but were soluble in methyl and ethyl alcohols and in pyridine. They obviously contained the experimental material.

In the sections these cells had a coarsely vacuolated cytoplasm, which lent to them the foamy appearance characteristic of the cells in lipoid storage diseases (fig. 3, *6;* fig. 4, *9, 10* and *11*). The cytoplasm of the smaller cells appeared fluffy rather than actually vacuolated (fig. 4, *10*), while that of the bigger cells was riddled with large vacuolar spaces of different sizes. It was slightly basophilic, dense or cloudy, and often coarsely striated. It stained deep blue with Mallory's aniline blue mixture, and the striations became intensified (fig. 4, *14*). The nuclei were usually eccentrically placed and varied from reticular in the smaller cells to stippled or homogeneous in the largest ones. Binucleated or giant cells were rarely observed.

The tests for iron (prussian blue) revealed large amounts of unmasked iron between the vacuoles but practically no masked iron. The vacuolar spaces themselves did not stain but were usually rimmed with bright blue cytoplasm. Discrete dark blue deposits occurred occasionally, but usually the cytoplasm was diffusely stained, or striated, with blue, which varied in intensity from pale to azure somewhat according to site. The macrophages in the spleens and the marrows generally stained the darkest, while those in the livers rarely contained more than traces of iron.

These cells occurred in tremendous numbers in the spleens. The spleens were enlarged in all dimensions, the size being relatively proportional to the daily dose of experimental material and the total amount given. With more extreme involvement they were exceedingly firm, rose colored and gritty on section and had unusually prominent trabeculae. The sections revealed lymphoid depletion ranging from an almost normal appearance in the spleen of rabbit 1 to practically complete obliteration of the corpuscles in the more involved spleens (fig. 3, 3). The cords were swollen and densely packed with the characteristic macrophages in almost direct proportion to the lymphoid depletion (fig. 3, *4, 5* and *6*). In rabbit 1, which received the smallest dose, the reticular cells were merely hypertrophied and

12. Tompkins (footnotes 1 and 2).

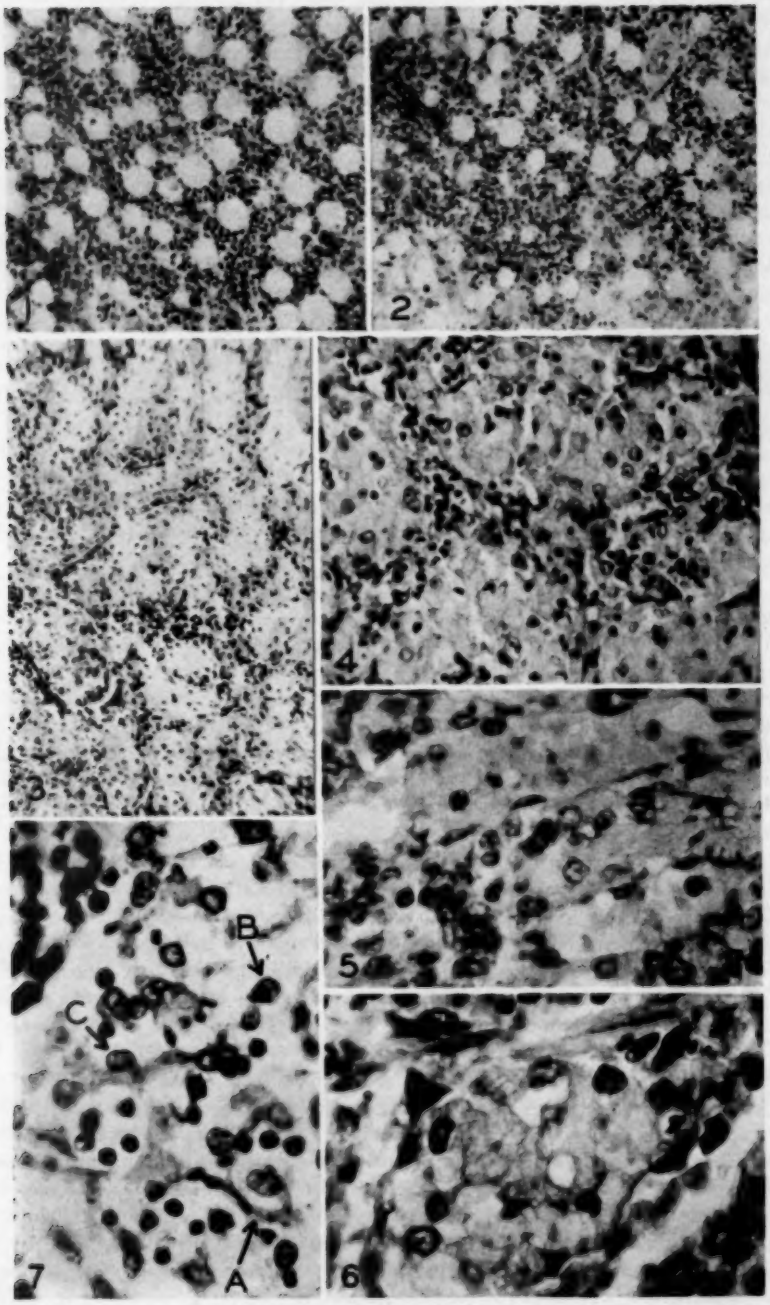

Figure 3

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prominent; foam cells completely replaced the reticular cells in the animals which received the larger doses (fig. 3, *4*). The endothelium of the sinusoids appeared normal (fig. 3, *5* and *6*). The sinusoids themselves were compressed between the swollen cords to degrees which lent a characteristic mosaic appearance to the tissue (fig. 3, *3* and *4*). They contained macrophages rich in unmasked iron and similar to those of the cords, but relatively few other cells, and almost no free debris.

Macrophages also occurred in surprising numbers in the lungs. Although the organs appeared normal grossly, the supravitally stained scrapings showed that they were literally swarming with macrophages, and the sections revealed definite pathologic change. In the lungs of the animals which received the largest daily doses the macrophages were similar supravitally to the characteristic macrophages elsewhere. In the lungs of the animals which received smaller daily doses, however, about half of the macrophages contained small uniform vacuoles, in contrast with the large vacuoles in the macrophages elsewhere. The small size and the uniformity of the vacuoles in these cells were suggestive but not typical of epithelioid cells. Though small, the vacuoles were never extremely fine and dustlike as are those in epithelioid cells, and were rarely collected into typical rosettes. Moreover, the cells rarely contained refractive droplets of fat and were never multinucleated. This type of staining is characteristic of the cells found in supravital scrapings of any normal lung, but the numbers of such cells were far in excess of the normal. They occurred almost exclusively in the lungs of the animals given the smallest daily doses of the experimental material. On the basis of transitions, therefore, it is believed that they represent advanced stages of the characteristic macrophages found elsewhere. The sections of the lungs revealed

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EXPLANATION OF FIGURE 3

1, marrow from rabbit 4. Central marrow from the femur, showing marked myeloid hyperplasia. Kingsley's technic; × 115.

2, marrow from rabbit 13. Central marrow from the femur, showing marked myeloid hyperplasia. Kingsley's technic; × 115.

3, spleen from rabbit 9. The pulp cords are crowded and distended with foam cells. The lymphoid tissue is practically obliterated, and the sinusoids are greatly compressed. A characteristic mosaic appearance results. × 115.

4, spleen from rabbit 13. The remarks made in connection with *3* apply also here. The pale foam cells of the cords, the compressed sinusoids and the mosaic appearance are striking. × 331.

5, spleen from rabbit 11. The remarks made in connection with *3* and *4* apply in general here. The experiment was terminated sooner than the experiments represented in *3* and *4*, and the cords were not so widely distended. Foam cells are shown developing in a cord between two sinuses and are in contrast with the normal-appearing endothelial cells. × 636.

6, spleen from rabbit 9. Higher magnification of *3*. The coarse, uneven vacuolation of the foam cells and their size and character are evident in contrast with the endothelial cells. The uniformity in type of the cells in the cords is striking. × 636.

7, mesenteric lymph node from rabbit 4. The sinusoids contain foam cells and many large nongranular and nonvacuolated cells, which are deeply basophilic. The reticular cells are enlarged and fluffy or slightly vacuolated. Arrow *A* points to a foam cell; *B*, to a basophilic cell, and *C*, to a reticular cell. × 636.

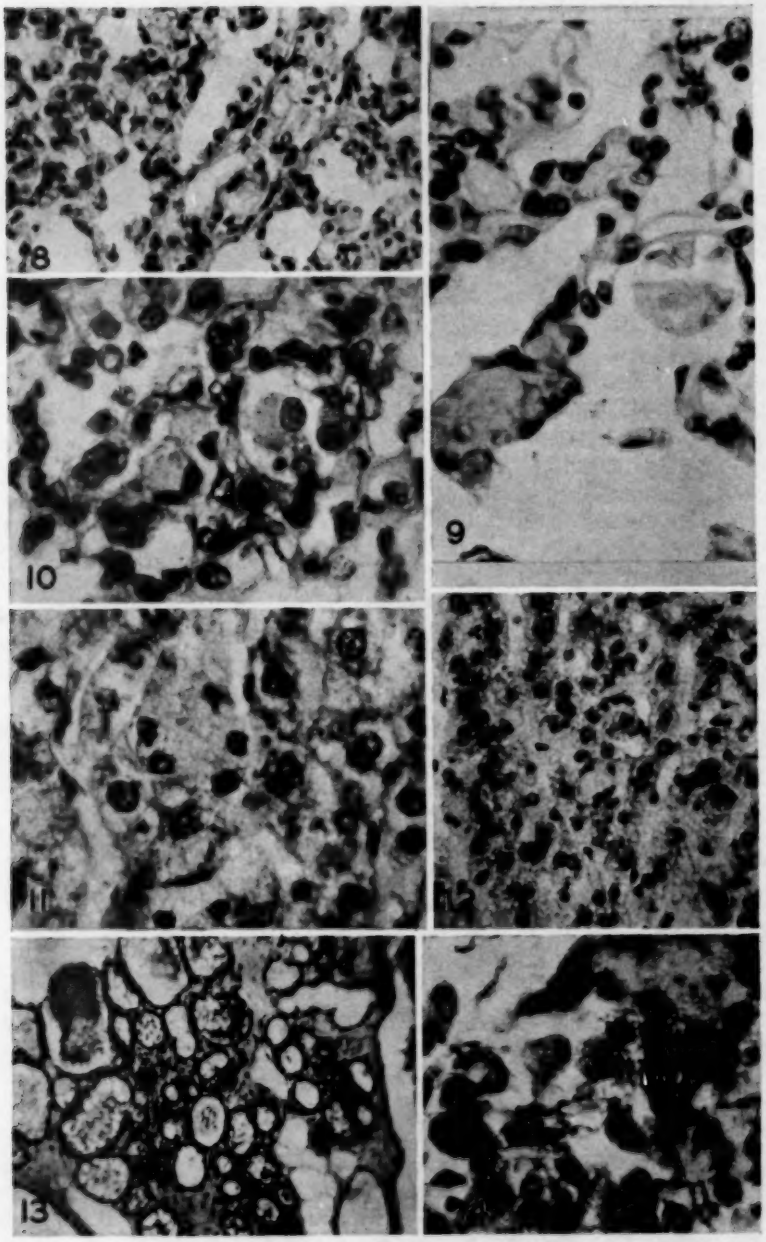

Figure 4

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generalized diffuse septal infiltrations and concomitant restrictions of the alveolar spaces, often to astonishing degrees (fig. 4, *8* and *10*). The broad septal areas were loosely packed with cells, which varied from coarsely vacuolated ones (fig. 4, *9*) to finely vacuolated, or fluffy, ones which could not be differentiated definitely from the epithelial cells (fig. 4, *10*). Similar cells occurred frequently in the pulmonary alveolar spaces of the rabbits which received the largest doses, but infrequently otherwise.

Macrophages were also found in great abundance in the livers. These organs were voluminous, pale and gritty on section, and the periportal areas were prominent. In the supravitally stained scrapings, clusters of the characteristic macrophages were often found surrounding the parenchymal cells. In the sections they appeared as clumps of large foam cells within the sinusoids, in numbers relatively proportional to the daily doses (fig. 4, *11* and *12*). The endothelial cells about these clumps appeared normal except in the rabbits which received the largest doses, in which they were often moderately foamy. The parenchymal cells when stained supravitally were strikingly free of fat and peculiarly homogeneous and structureless except in animals given the largest doses or in those that had shown evidence of intolerance to the injections preceding death (rabbit 6). In this respect the observations in the liver are similar to those after injections of lecithin; i. e., fat was found in the hepatic parenchymal cells only in those rabbits which received excessive doses.

The lymph nodes appeared normal grossly, but supravitally stained scrapings revealed the characteristic macrophages in numbers somewhat proportional to the daily dose and, in addition, unusual numbers of large young cells which were nonvacuolated and contained large rod-shaped mitochondria. In sections the latter cells were found free in the sinusoids of both the cortex and the medulla; the nuclei were reticular, and the nonvacuolated cytoplasm varied from deep to pale

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EXPLANATION OF FIGURE 4

8, lung from rabbit 13. A widespread diffuse foam cell infiltration occurred in all septums and to a lesser extent in the alveolae. As a result, the alveolar spaces are markedly constricted. × 331.

9, lung from rabbit 4. Large clumps of foam cells distend the septums and occur infrequently in the alveolae. × 636.

10, lung from rabbit 9. Foam cells occur in great numbers in the septums and alveolae. The alveolar spaces are constricted, and the differentiation of epithelium from foam cells is uncertain. × 636.

11, liver from rabbit 9. Clumps of foam cells fill the sinusoids. A large clump shows in the center of the picture. These cells are in striking contrast with the slender endothelial cells, which appear entirely normal. The hepatic parenchymal cells appear normal and show strikingly little vacuolation. × 636.

12, liver from rabbit 5. Many clumps of pale foam cells occur in the sinusoids between the cords of normal-appearing, nonvacuolated parenchymal cells. × 331.

13, mammary gland from rabbit 10. Hyperplasia and lactation were present. The ovaries contained large follicles and generalized interstitial luteinizations, but corpora lutea were not present. × 75.

14, subcutaneous area from a mouse given an injection of an experimental mixture of kerasin and sphingomyelin. Mallory's connective tissue stain. The cells stained deep blue, and striations of the cytoplasm are prominent. × 636.

blue. These cells were similar morphologically and in site to cells observed in the nodes of animals given lecithin intravenously. Unlike the macrophages in other areas, those in the nodes frequently contained fine yellow granules in addition to the characteristic deposits. The reticular cells in the nodes of the animals which received the largest doses were hypertrophied and similar to the reticular cells in the spleens of the animals which received the smallest doses (fig. 3, *7*).

The thymuses were voluminous, very white and somewhat firmer than normal. The reticular cells were markedly increased and moderately hypertrophied, especially those of the medulla, but frank foam cells were infrequent. They were like the reticular cells in the nodes. These changes were proportionate more to the total amount of experimental material given than to the size of the daily dose.

The adrenal glands were unusually small, often smaller than those of the guinea pig. In this they resembled the adrenal glands of rabbits given lecithin, but unlike those, they revealed no evidence of abnormality of the inner part of the zona fasciculata or of the zona reticularis. Foam cells occurred rarely in the sinusoids.

The kidneys appeared more abnormal grossly than was supported by the sections. Except in the 2 rabbits which received the smallest doses, they were pale and voluminous, and the cut surfaces had an opalescent glassy appearance which gave the impression of china or wax models. The two parts of the outer zone of the medulla merged with each other and with the cortex to an unusual degree. Supravitally stained scrapings revealed unusual numbers of coarse red droplets in the tubular cells. The sections, however, revealed scattered foam cells between the tubules and many clumps of them within the glomerular tufts as the only abnormalities. As Ferraro and Jervis observed after injections of sphingomyelin, foam cells were never found free in the glomerular spaces.

The mammary glands of rabbits 1, 2, 8, 9, 10 and 11 were hypertrophied and exhibited various degrees of secretory activity (fig. 4, *13*). In each case, general interstitial luteinization of the ovaries was present, and the follicles were large, but there were no corpora lutea. The mammary glands of the remaining animals were inactive, the ovaries contained various-sized follicles, and the ovarian interstitial cells were slender and spindle shaped. These findings suggest that the experimental substance was capable of stimulating lactation if ovarian activity was at a stage to initiate hyperplasia of the mammary tissues, but was otherwise inactive in this respect.

The tissues from the mice given the same experimental material for variable periods were entirely comparable to those described in the rabbits.

COMMENT

It is obvious that intravenous injections of the ether-insoluble fraction of the lipoids from brains, containing both the sphingomyelins and the galactolipids, stimulated the production of macrophages in the same sites throughout the organism as experimental injections of the phosphatides sphingomyelin [8] and lecithin,[7] and that these sites are the same as those involved in the lipoid storage diseases, namely, the reticuloendothelial system in the marrow, the liver, the nodes and the spleen with more or less obliteration of the malpighian corpuscles and compression of the splenic sinusoids and with variable degrees of capillary accumulation of the characteristic macrophages throughout the organism.

The macrophages differed considerably from those called forth by the phosphatide lecithin in that the latter were smaller and contained iron in a masked form which was apparently so bound with lipoids that the cytoplasm was not vacuolated after treatment with fat solvents. In most respects the cells were similar to those which Ferraro and Jervis [8] obtained with injections of the phospholipid sphingomyelin but differed from them in the irregularity in size of vacuoles, the frequency of striations in the cytoplasm and the abundant content of unmasked iron. In these very respects they resembled the cells characteristic of Gaucher's disease.[13] When stained supravitally, they were identical with the cells called forth locally by subcutaneous injections of mixtures of sphingomyelins and galactolipids.[2] These were also found to resemble the cells of Gaucher's disease.

Except for these differences in character of the invading macrophages, therefore, the organic lesions in these experiments, in those of Ferraro and Jervis with sphingomyelin, in those of Tompkins with lecithin and in the lipoid storage diseases are essentially similar. The reactions of the lungs and of the hemopoietic centers represent exceptions to this generalization. Ferraro and Jervis obtained extensive foam cell infiltrations of the lungs and marked reductions of alveolar space following injections of sphingomyelin. The pulmonary involvements in the present series of experiments were entirely comparable. In addition, periarterial accumulations of eosinophils frequently occurred. Macrophage infiltrations of the lungs following injections of lecithin, on the other hand, and restrictions of alveolar space were inconspicuous. Periarterial accumulations of eosinophils, however, were present. From the fact that the two phosphatides caused such different pulmonary changes it seems probable that the differences in pulmonary involvement under these three experimental conditions reflect merely mechanical factors concerned with differences in size and character of the invading macrophages. However, since sphingomyelin was a component of the experimental mixture used in the present series of studies as well as in the material used by Ferraro and Jervis, it is possible that the similarities of pulmonary reaction in these 2 cases represent a specific response to that lipoid.

The reactions of the hemopoietic centers in reference to the three sets of experimental injections varied markedly. The present series of injections caused sustained increases of the circulating granulocytes and lymphocytes and less regularly of the monocytes, with corresponding hyperplasia of the myeloid centers of the marrow at the late myelocytic and mature polymorphonuclear levels, but without foci of extramedullary

13. Bloom, W.: Am. J. Path. **1**:595, 1925.

hemopoiesis. Intravenous injections of the phospholipid lecithin caused similar, though less intense, increases of the circulating lymphocytes and monocytes but, on the other hand, had little effect on the granulocytes. Since phospholipids were present in both cases, it is probable that the increases in circulating lymphocytes and also, less regularly, in monocytes represent a characteristic response to repeated intravenous administration of phospholipids. It is significant in this respect that increased activity of the lymph nodes and depletion of the splenic nodules occurred in both of these experiments and that the latter also occurred in the experiments which Ferraro and Jervis carried out with sphingomyelin.

The marked hyperplasia of the granulocytic centers, however, which was evident in the blood and marrow in the present series of experiments was lacking in the experiments with lecithin, although extramedullary hemopoiesis occurred with formation of granulocytes as well as of erythrocytes. Ferraro and Jervis did not report studies of the blood after injections of sphingomyelin, but they examined the marrow and noted replacement of fat by the characteristic foam cells; they made no comment as to either myeloid or erythroid hyperplasia. It is probable, therefore, that the sustained stimulation to granulocytic activity in the present experiments is related to the component of galactolipids in the experimental material. In line with this probability is the fact, which was discussed earlier, that while lecithin and sphingomyelin acted alike in stimulating local macrophage infiltrations after subcutaneous injections, similar injections of galactolipids elicited only neutrophils and fibrous tissue.[1] It is possible, from the method of preparation, that the biologic mixture of lipoids used in the present studies carried an impurity capable of chemotactic effect on the granulocytes. It is believed that this possibility is ruled out by the filtration of the experimental material after solution in alcohol and chloroform, by the reactions of the blood cells following single injections of the same material after treatment for removal of possible water-soluble impurities and by tests for specific impurities. These data are included in the report of the reactions of the blood cells following individual injections of the experimental material.[9b]

In the experiments with lecithin, in contrast with the present series, evidences of hyperactive erythropoiesis were present in the blood, marrow and spleen, and the macrophages, though filled with masked iron, contained little unmasked iron. While anemia of a similar grade developed in the present experiments and the macrophages also contained iron, evidences of increased erythropoiesis were not obtained and the iron was largely unmasked. It is unfortunate that the experiments of the present series were carried out before those with lecithin and did

not include detailed studies of the erythrocytes to serve as a more comprehensive basis of comparison in this respect. Ferraro and Jervis, likewise, made no note of increased erythropoiesis in the tissues following injections of sphingomyelin, and they failed to find iron in the macrophages. It was suggested that the anemia in the experiments with lecithin was due to the influence of that phospholipid on the resistance of erythrocytes coupled with excessive trauma within the compressed and crowded splenic sinusoids. The splenic sinusoids in the present experiments were even more compressed, yet the evidence at hand is not indicative of excessive destruction and increased production of erythrocytes, but points rather to low grades of anemia similar to those often met in conditions, including Gaucher's disease, in which there is splenic enlargement but no appreciable change in the resistance of the erythrocytes. It seems probable, therefore, that the mixture of galactolipids and sphingomyelins used in these experiments exerted little effect on the resistance of the erythrocytes and that they were able to withstand the crowded conditions in the spleen to the same degree as normal cells.

It is obvious, therefore, that intravenous injections of the biologic mixture of galactolipids and sphingomyelins used in the present series of experiments resulted in widespread infiltrations of macrophages which were like those of Gaucher's disease and in tissue changes characteristic of the lipoid storage diseases in general. In addition, they caused increases in the circulating lymphocytes and granulocytes and changes in the centers of formation of those cells which seem attributable to the phospholipid and galactolipid components of the mixture, respectively.

SUMMARY

Repeated intravenous injections of the ether-insoluble lipoids of beef brain (biologic mixture of galactolipids and the sphingomyelin group of phospholipids) cause: (*a*) an increase in the number of white blood cells dependent on increases in the numbers of neutrophils and lymphocytes and less regularly in that of monocytes; (*b*) a generalized infiltration of the reticuloendothelial organs with macrophages similar to those obtained locally after subcutaneous injections of experimental mixtures of galactolipids and phospholipids, the macrophages having the characteristics of the foam cells found in Gaucher's disease; (*c*) hyperplasia of the marrow with increased myelopoiesis at late levels of maturation, increase of the myeloid-erythroid ratio and infiltrations of the characteristic macrophages; (*d*) splenomegaly associated with massive infiltrations of the characteristic macrophages, depletion of the malpighian corpuscles

and constriction of the sinusoids; (*e*) diffuse pulmonary infiltrations of the characteristic macrophages with restrictions of alveolar space.

The results differ from the results of Ferraro and Jervis [8] with sphingomyelin by slight morphologic differences in the invading macrophages and by stimulation of the granulocytic centers. The results differ from those obtained by me with lecithin in the appearance of the invading macrophages and the presence of unmasked rather than masked iron in them, in the stimulation of the myeloid rather than the erythroid centers, in the absence of extramedullary hemopoiesis and in the presence of diffuse infiltrations of macrophages in the lungs. The organic lesions are otherwise similar to those following injections of the phosphatides alone and to those of the essential lipoid storage diseases.

CELLULAR ORIGIN OF BRONCHIAL ADENOMA

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During the past fifteen years a number of publications have appeared describing a special group of tumors which develop in the large bronchi in youth and middle age. These are characterized by slow growth, by formation of polypoid projections which obstruct the bronchi and, in some instances, by penetration of the bronchial wall and the formation of more or less massive tumor in the surrounding lung. Metastases have been reported in the regional lymph nodes, the liver and the bone marrow (Castleman [1]; Adams, Steiner and Bloch [2]), but they are uncommon. These tumors have a distinctive morphologic appearance which has been described by many in a superficial fashion but by only a few with care and after good fixation and staining. The majority of writers have supposed that they have developed from the mucous and serous glands of the mucosa or from their ducts. This has not satisfied all observers, however, because the tumors seldom form gland spaces and it is rare indeed for their cells to secrete mucin. Womack and Graham [3] therefore turned to the developmental stages of the bronchi and supposed that the tumors came from undeveloped bronchial buds. They supported this hypothesis by their report of the finding of bone and cartilage in their tumors, but Tracy Mallory [4] pointed out that these tissues may form in pulmonary tumors as a result of metaplasia of the stroma and that the fragments of cartilage found in them may be remnants of bronchial cartilage surrounded by infiltrating tumor strands. Among the 20 specimens of bronchial adenoma studied in the Columbia University Laboratory of Surgical Pathology none shows any bone or cartilage forming an integral part of the tumor, and I agree with Mallory that the hypothesis of Womack and Graham has insufficient foundation to make it acceptable for tumors of this group. That tumors can form as the result of such developmental faults is illustrated in a case reported by Rosenblum and Klein [5] in which a polypoid tumor grew in the right main bronchus of an 11 year old boy and was made up of ductlike structures lined with ciliated epithelial cells and mucous glands.

The most careful and accurate histologic study of the tumors under discussion has been made by Hamperl.[6] He described 9 and divided them into two groups. He expressed the belief that 2 of the tumors had many features in common with the cylindromatous form of the mixed tumor of mucous and salivary glands and these he called cylindroma. He supposed that they were derived from the mucous glands of the bronchi. This is evidently a rare type. Jacob and his associates [7] have recorded another such tumor, and I have seen sections from a tumor of this type, although they are no longer in my possession. Clinically these tumors cannot

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1. Bronchial Adenoma, Cabot Case 26171, New England J. Med. **222**:721, 1940.
2. Adams, W. E.; Steiner, P. E., and Bloch, R. G.: Surgery **11**:503, 1942.
3. Womack, N. A., and Graham, E. A.: Arch. Path. **26**:165, 1938.
4. Bronchial Adenoma, Cabot Case 27511, New England J. Med. **225**:983, 1941.
5. Rosenblum, P., and Klein, R. I.: J. Pediat. **7**:791, 1935.
6. Hamperl, H.: Virchows Arch. f. path. Anat. **300**:46, 1937.
7. Jacob, P.; Delarue, J., and Gaultier, M.: Bull. Assoc. franç. p. l'étude du cancer **28**:408, 1939. Jacob, P.; Delarue, J.; Huet, P., and Depierre, R.: Bull. et mém. Soc. méd. d. hôp. de Paris **57**:95, 1941.

be distinguished from those of the second group, but it seems wiser to regard them as representing a different histologic type identical with the mixed tumor of salivary glands.

Hamperl's second group had the more common morphologic character, which he described in great detail. The tumor cells formed solid cords and strands, which tended to anastomose. Most of them were rounded or polygonal, and in all but 1 of his tumors, had acidophilic granules. In this seventh tumor the granules were amphoteric. Sometimes the tumor cells were elongated and resembled truncated cones. When such cells were arranged in a cord with their axes at right angles to the axis of the cord and with each alternate cell pointing in a direction opposite to its neighbor, a palisaded effect was produced. Palisaded cells sometimes outlined the larger masses of rounded cells. Occasionally the rounded cells surrounded a tiny space containing a droplet of mucoid material. The cell groups and cords frequently tended to be drawn away from the fibrous framework, leaving a free space between them. Almost all of these features have been recorded by Masson [8] as characterizing the carcinoid tumors of the appendix, and Hamperl was struck by the marked resemblance between the two types of tumors. There are differences, however, to which he called attention. The carcinoid of the gastrointestinal tract has granules which, after fixation in dilute solution of formaldehyde or Bouin's fluid, can be blackened with ammoniacal silver nitrate. The granules of bronchial adenoma are unaffected by the same treatment. The cells of bronchial adenoma, according to Hamperl, occasionally contain mucus which mucicarmine will redden; this is not true of the cells of gastrointestinal carcinoids. Finally, 2 of the tumors described by Hamperl contained special cell forms to which he has given the name "onkocytes." These cells are characterized by their voluminous acidophilic granular cytoplasm, which makes them differ from the other tumor cells. My observations on 20 tumors confirmed all of Hamperl's findings except in regard to the palisaded cells. I could find small ribbons of four or five cells arranged in this fashion but never any long ones. It seems proper also to make one addition to Hamperl's description. As pointed out by Zamora and Schuster [9] and others, and confirmed in this series, some of these tumors have an exceedingly vascular stroma and bleed freely and repeatedly. This is not a characteristic of the carcinoid tumors of the gastrointestinal tract.

Because of the resemblance to the gastrointestinal carcinoids Hamperl felt that the tumors in question should be called bronchial carcinoids, but he did not suggest for them a possible cellular origin and left this question unanswered.

It seemed remarkable to me that although Hamperl found oncocytes in 2 tumors diagnosed as bronchial adenoma, he did not investigate the bronchial mucosa to determine whether or not any of these cells could be found in it. Hamperl [10] is the originator of the term "onkocyte" and has expended much effort in a study of this peculiar cell type and the tumors derived from or containing oncocytes. According to him, oncocytes are epithelial cells which resemble those of the organ in which they are found but are larger and are distinguished by having in their cytoplasm distinct, markedly acidophilic granules and nuclei which either look like the nuclei of the surrounding cells or which may be more deeply stained or even appear pyknotic. He has found such cells in the salivary glands, the anterior and posterior lobes and the stalk of the hypophysis, the thyroid and parathyroid glands, the pancreas, the liver, the uterine tube and the testis. He has described

8. Masson, P.: Ann. d'anat. path. **1**:3, 1924.

9. Zamora, A. M., and Schuster, N.: J. Laryng. & Otol. **52**:337, 1937.

10. Hamperl, H.: (*a*) Virchows Arch. f. path. Anat. **282**:724, 1931; (*b*) Ztschr. f. mikr.-anat. Forsch. **27**:1, 1931; (*c*) Virchows Arch. f. path. Anat. **298**:327, 1936.

adenoma composed of oncocytes in salivary glands and has found these cells in adenolymphoma (papillary cystadenoma lymphomatosum), simple salivary gland cysts and 1 mixed tumor of salivery glands. He found oncocyte adenoma in the anterior and posterior lobes and the stalk of the hypophysis. He expressed the belief that the oxyphilic cells in adenoma of the parathyroid gland are oncocytes, and he expressed the view that the acidophilic granular cell tumor of the thyroid gland is a tumor of oncocytes. He referred to the oncocytes of the thyroid gland as Askanazy cells and was apparently unaware that in American literature they are called Hürthle cells and the tumors Hürthle cell tumors (Ewing [11]; Haagensen [12]). Finally, as already stated, he described oncocytes in bronchial "carcinoids" (i. e., adenoma).

PROCEDURE IN PRESENT STUDY

With the assistance of Dr. Robert C. Horn, I investigated the large bronchi of 3 mice, 2 human fetuses and 10 human adults varying in age from 19 to 80 years. All the human material was obtained at autopsies on persons who had died without primary bronchial disease except 1 adult, who had bronchiectasis. The fetuses were all aborted ones. Of the remaining 9 adults, 3 had lungs that were entirely normal, 2 had terminal edema, 1 had a pulmonary embolus, 2 had terminal pneumonia and 1 a terminal bronchitis.

The large bronchi were fixed in Bouin's fluid, and one set was immersed ninety-six hours in 10 per cent ammoniacal silver nitrate solution (Fontana's fluid, modified by Laidlaw) and counterstained with Masson's aniline blue, ponceau, acid fuchsin trichrome stain.[13] A second set was stained with Masson's trichrome stain without silver.

OBSERVATIONS

In none of the silver preparations were any cells found containing blackened granules. Of the sections prepared with Masson's trichrome stain alone, none from murine bronchi or human fetal bronchi showed cells with acidophilic granules. But all of the adult human bronchi showed scattered groups of cells with acidophilic granules both in the glands and in their ducts but not among the surface ciliated epithelial cells. These cells have all the features which Hamperl indicated were characteristic of his "onkocytes." They are the same size or somewhat larger than the neighboring cells except when the latter contain mucus; they have distinct regularly spaced cytoplasmic granules made bright red with fuchsin, and they have nuclei which either resemble those of adjacent cells or are more deeply stained and sometimes pyknotic. They occur in small groups, usually at wide intervals. Mucin has not been found in any of them. These cells are not described in the mucosa of bronchi by Heiss [14] or by any of the other histologists so far as I am aware.

In order to compare these cells with those composing the bronchial tumors, I photographed two acini with oncocytes in them from bronchial glands obtained at autopsies and, at exactly the same magnification, cells of three bronchial tumors (adenoma). In each case Masson's trichrome stain was used. From these photographs the composite picture illustrating this paper was compiled. It shows that the nuclear structures are comparable both in size and nucleolar arrangement. The tumor cells seem somewhat smaller and their granules not quite so regular. In color the tumor cell granules are not as deeply red. In spite of these differences the resemblance between the two is still quite striking, sufficiently so to warrant

11. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 954.

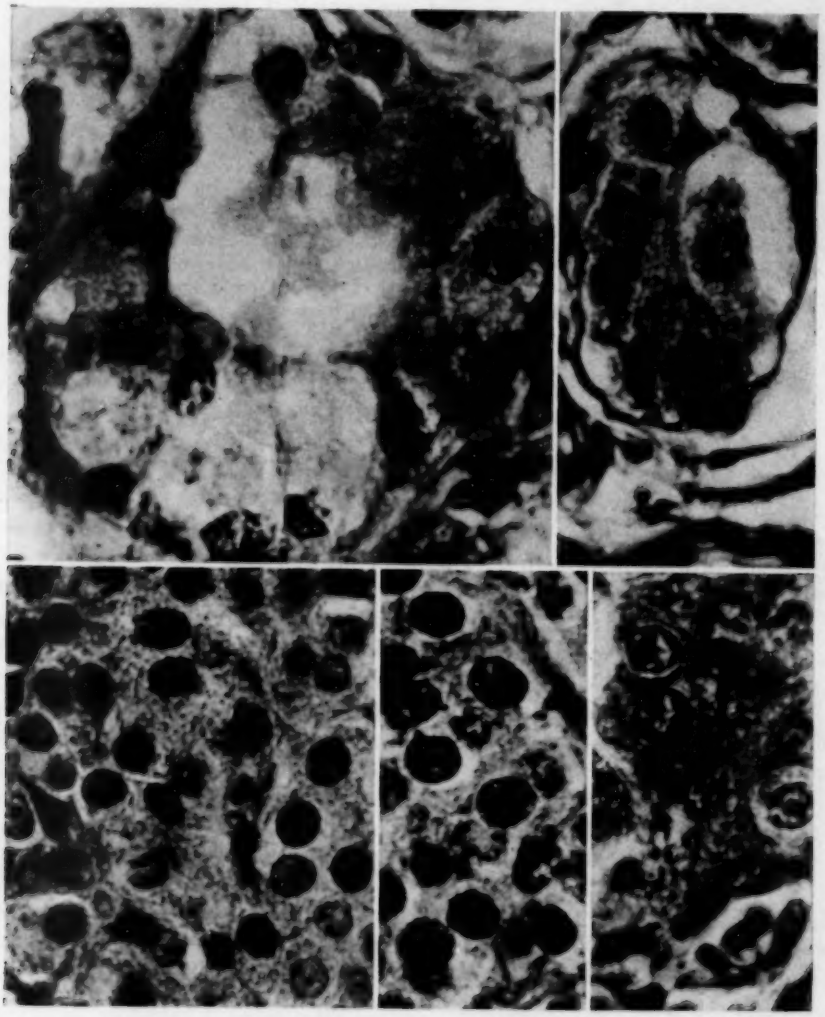
12. Haagensen, C. D.: Am. J. Cancer **15**:2063, 1931.

13. Masson, P.: J. Tech. Methods **12**:75, 1929.

14. Heiss, R.: Der Atmungsapparat, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1936, vol. 5, pt. 3, pp. 749-750.

serious consideration of these cells of the bronchial glands with their acidophilic granules as a possible source from which the bronchial tumors are derived.

What are these oncocytes? Hamperl [10a] pointed out that they were first recognized in salivary glands, in 1897, by Schaffer, who called them granular swollen cells. Zimmermann [15] described them again, called them pyknocytes and discussed their function. He rejected Schaffer's idea that they are degeneration



The top row shows acidophilic granular cells (oncocytes, pyknocytes) in the mucous and serous glands of the main bronchi of an 80 year old woman (left) and a 37 year old man (right). The bottom row shows the tumor cells of three different specimens of bronchial adenoma. All sections were stained with Masson's trichrome stain. The composite picture is slightly reduced from a magnification of 1,330.

forms and Pischinger's supposition that they are "reserve" cells and came to the conclusion that their function is unknown. Beyond the fact that he recognized them

15. Zimmermann, K. W.: Die Speicheldrüsen der Mundhöhle, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 5, pt. 1, pp. 128-129.

in other parts of the body besides the primary and accessory salivary glands, Hamperl, too, has been unable to determine their functions if any. The investigations in this laboratory have established their presence in the bronchi of adults and their absence in fetal bronchi but have thrown no light on their functions.

Are the tumors diagnosed as bronchial adenoma derived from these cells? Certainly the two bronchial tumors described by Hamperl [6] in which oncocytes predominated must have been derived from these cells. But the large majority of the tumors grouped as bronchial adenoma are composed of cells which are not faithful reproductions of oncocytes, although they have certain resemblances to them. In spite of this, the other cells in the bronchial mucous membrane are very different indeed from the tumor cells, and one is almost forced to choose the oncocyte as the probable cell of origin, for no other cell, either in the bronchus or in the surrounding lung, offers itself as a possible candidate. The location of oncocytes in the bronchial glands and their ducts but not among the ciliated lining cells offers indirect support for this conception, since all observers are agreed that the tumors in question only secondarily involve the lining cells and could not be derived from them.

As there still remains an element of doubt about the cellular origin of this group of bronchial epithelial tumors, it does not seem proper to enter into a protracted discussion of names. There are arguments pro and con for all of the current designations. At present I use names which I believe are most widely current and therefore familiar to the largest number. I call the tumors under discussion adenoma but reject the descriptive adjective "benign" because of the aggressive infiltrative growth displayed by many of them and the rare occurrence of metastases. I recognize in addition the following: mixed tumors derived from the bronchial mucous and serous glands and similar to the mixed tumors of salivary glands, mucous gland adenoma and finally the hamartoma described by Rosenblum and Klein.[5] There are in addition fibroma, chondroma, lipoma, papilloma, lymphoma and a thyroid gland tumor as noted by Lindgren,[16] but these could not be confused with the group under discussion and are mentioned only to complete the roster of bronchial tumors other than carcinoma, sarcoma and metastases.

SUMMARY

The peculiar cells with acidophilic granules called oncocytes or pyknocytes have been demonstrated among the mucous and serous glands of adult human bronchi and their ducts. The relationship of these cells to the cells of bronchial adenoma is discussed, and although no conclusion is reached, it is considered possible that they may be the stem cells for tumors of this type.

16. Lindgren, A. G. H.: Acta oto-laryng. **27**:183, 1939.

BACILLUS COLI PNEUMONIA

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AND
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The problem whether Bacillus coli can produce pneumonia arose for us when bronchopneumonia was found at an autopsy in which the evidence pointed to this organism as the etiologic agent. The patient was a middle-aged woman with chronic glomerulonephritis who died in uremia. Material taken from the lung at autopsy, which was done almost immediately after death, yielded on culture a heavy growth of B. coli. Sections of lung showed large numbers of both intracellular and extracellular gram-negative bacilli. Because no other organisms except rare cocci were seen in the lesion, and because such an incitant as a virus, a toxin or a chemical could be excluded on the basis of the type of anatomic response, it seemed reasonably certain that the pneumonia was produced by B. coli.

The question of whether B. coli can cause pneumonia arose again shortly afterward when the sputum of 2 patients with pneumonia gave pure cultures of B. coli on repeated examinations.

A survey of the literature was of little aid in answering this question. Several cases have been presented as instances of B. coli pneumonia, but in most of these there was no definite proof that the pneumonia was caused by this organism, the conclusions having been based on inadequate evidence. Only one set of experiments on animals was found in which organisms believed to be B. coli were intratracheally inoculated. These experiments were done by Kreibich,[1] in 1896; he produced bronchopneumonia in rabbits, but the methods he used to identify the cultures actually did not exclude some of the other gram-negative bacilli.

In view of the paucity of information in the literature and the lack of well controlled experiments along this line, the present study was undertaken to determine whether B. coli can cause pneumonia when introduced into the trachea of the rabbit.

REVIEW OF THE LITERATURE

The articles are reviewed in some detail because there is a lack of definite identification of the organisms described as "B. coli" and because some of the cases reported were later quoted as definite instances of B. coli pneumonia. In most cases detailed description of the pulmonary lesions was not recorded, but the impression was given that these lesions corresponded to those of the usual type of bronchopneumonia, consisting of a response by fibrin and polymorphonuclear leukocytes.

Sevestre [2] in 1887 noted that bronchopneumonia developed in many infants with infectious diarrhea. After studying many of these patients clinically and at autopsy, he decided that the bronchopneumonia was of intestinal origin. Unfortunately, he did not make bacteriologic studies. To fill this lack, an associate of his, Lesage,[3]

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1. Kreibich, K.: Beitr. z. klin. Med. u. Chir., 1896, no. 13, p. 1.
2. Sevestre: Bull. et mém. Soc. méd. d. hôp. de Paris **4**:12, 1887.
3. Lesage: Bull. et mém. Soc. méd. d. hôp. de Paris **9**:28, 1892.

studied a similar group of infants bacteriologically. In the postmortem examinations he found B. coli in all organs; from the lungs of those infants who also had bronchopneumonia he isolated B. coli in pure cultures. He expressed the belief that the B. coli came from the intestine. The organism that Lesage called B. coli was evidently a gram-negative rod. Since he did not mention sugar reactions, one cannot exclude the other gram-negative bacilli, but in all probability he was dealing with B. coli.

In a discussion of Lesage's paper, Sevestre [3] quoted Widal and Chantemesse,[4] who stated that in many cases of bronchopneumonia they found an organism which had all the characteristics of B. coli. We were unable to obtain the original article. The organism they described was most likely B. coli, since in an article published the previous year (1891) dealing with B. coli infections these authors described the use of lactose to differentiate between B. coli and Bacillus typhosus.[5]

Welch [6] stated:

> . . . I have suspected that the colon bacillus may be the cause of lobular pneumonia, as in several cases this organism has been found in large number and in pure culture in congested, edematous and inflamed areas in the lungs. It has also been frequently associated with fatty degeneration of the kidneys, but neither in this nor in the pulmonary affection is there any conclusive evidence that the presence of the bacilli has done the harm.

Fischer and Levy [7] described 2 cases of incarcerated gangrenous hernia complicated by bronchopneumonia. In each case cultures were made of the fluid found in the hernial sac at operation, as well as of the lungs and the peritoneal exudate at autopsy. The autopsies were done ten and twelve hours, respectively, after death. In the first case pure cultures of a bacillus were obtained from all three sites, while in the second the bacillus was found together with "Staphylococcus pyogenes albus." They described the bacillus as a short thick rod of sluggish motility. They believed it was "Bacterium coli commune." No Gram stain or sugar reactions were described.

We mention a case reported by Gilbert and Girode [8] because their report has been quoted several times in the literature as an instance of B. coli pneumonia. Actually, cultures of the lung showed a mixture of B. coli, "pneumococcus of Talamon" and Staphylococcus aureus.

Lemoine [9] described a case in which bronchopneumonia caused by B. coli was observed as a complication of intestinal obstruction. During life he punctured the lung and obtained a few drops of bloody fluid. Smears showed a large number of bacilli and only a few diplococci. The sputum showed the same organisms, in the same proportion. Cultures of the fluid obtained from the lung by puncture showed typical colonies of B. coli. The bacillus was motile and fermented lactose.

Kreibich [1] found a case of pneumonia caused by B. coli in a series of 28 cases of pneumonia studied at autopsy. The autopsy in this case was done sixteen hours after death. Smears of the lungs showed gram-negative bacilli; no other organisms were seen. Material from the lungs and the bone marrow yielded pure cultures of B. coli. Sections of the lungs were stained with hematoxylin and eosin, with Weigert's modification of the Gram stain and with Loeffler's methylene blue. The lungs showed extensive bronchopneumonia. The alveoli were filled with exudate, which consisted mainly of serum and polymorphonuclear

4. Widal and Chantemesse: Gaz. hebd. de méd., 1892, p. 16.
5. Chantemesse, Widal and Legry: Bull. et mém. Soc. méd. d. hôp. de Paris **8**:657, 1891.
6. Welch, W. H.: M. News **59**:669, 1891.
7. Fischer, F., and Levy, E.: Deutsche Ztschr. f. Chir. **32**:252, 1891.
8. Gilbert, A., and Girode, J.: Bull. et mém. Soc. méd. d. hôp. de Paris **8**:51, 1891.
9. Lemoine, G. H.: Bull. et mém. Soc. méd. de hôp. de Paris **11**:775, 1894.

leukocytes, as well as some erythrocytes and desquamated alveolar epithelium; fibrin was sparse. In the sections stained with Loeffler's methylene blue, numerous bacilli were seen. These bacilli were seen lying free in the exudate as well as within leukocytes and desquamated alveolar epithelium. No cocci were seen either in the sections stained with methylene blue or in those stained by the Weigert method. The bacillus was gram negative. No spores were seen. The Kitasato test for production of indol was positive. There was no liquefaction of gelatin in stab cultures. The only mention made of a sugar reaction was that the bacillus fermented 2 per cent *Zuckeragar* with production of gas. We cannot be certain which sugar medium was used. Although there is no mention made of the use of lactose medium, it seems quite likely that he was dealing with B. coli.

Kreibich then undertook some animal experiments, using the organism isolated from this case, as well as similar bacilli obtained from 3 cases of pneumonia in which both diplococci and B. coli were found. After being passed through mice, the bacteria were grown on agar slants and suspended in meat broth. He injected these suspensions into rabbits in doses varying from 0.5 to 2 cc. Of 12 rabbits inoculated intratracheally, 7 showed pneumonia; of 14 inoculated intrathoracically, 11 showed pneumonia. Most of the animals died within twenty-four hours. The lesions showed a preponderance of polymorphonuclear cells and serum in the exudate, within both the bronchi and the alveoli. Some erythrocytes and desquamated alveolar epithelium were also seen in the exudate. In some cases a hemorrhagic exudate predominated. In those rabbits which lived longer (two or three days) there were also thickening and small cell infiltration of the peribronchial tissues. A rabbit that received an intravenous injection of the suspension died the next day but showed no pulmonary lesion. Cultures of the lungs were positive for B. coli. Sections of the lungs examined microscopically showed varying numbers of bacilli, both extracellular and intracellular. In addition, numerous shorter rods and coccoid shapes were seen, which Kreibich considered to be degenerated forms of the same bacillus. He concluded that B. coli commune can produce pneumonia in man.

Pearce [10] described 5 cases of B. coli bronchopneumonia in a series of 128 cases of bronchopneumonia studied at autopsy. In 2 cases the pulmonary condition was a complication of typhoid fever; in 1, of gangrene of the lung; in 1, of pulmonary thrombosis, and in 1, of acute peritonitis.

Von Schrotter and Weinberger [11] described a case of B. coli pneumonia and laryngitis which they studied clinically.

Kemp [12] presented a case report of a patient who, after an operation for inguinal hernia, developed "double pyelitis, cystitis, double pneumonia, purulent bronchitis, two attacks of colitis and a myocarditis . . . all due to infection with the colon bacillus." The urine showed B. coli. Cultures of the sputum showed enormous numbers of colon bacilli and some streptococci. The patient recovered.

Hartshorn [13] stated:

> . . . In the respiratory tract the Bacillus coli is rarely the only cause of inflammatory processes. It has been found in pure culture in the sputum of patients suffering from pneumonia according to the case reports of Meara and Niles. At the Rockefeller Institute and at the Research Laboratory of the Board of Health there were no case records of pneumonia caused by the Bacillus coli alone.

10. Pearce, R. M.: Boston M. & S. J. **137**:561, 1897.
11. Von Schrotter, H., and Weinberger, M.: Wien. klin. Wchnschr. **21**:505, 1908.
12. Kemp, R. C.: Boston M. & S. J. **165**:819, 1911.
13. Hartshorn, M. W.: Am. J. Obst. & Gynec. **70**:482, 1914.

We were unable to find the case reports of Meara and Niles.

Felty and Keefer [14] described 28 cases of colon bacillus infection of the blood stream. In 5 of these there were metastatic lesions, including bronchopneumonia or septic infarction of the lung, caused by B. coli. These cases were proved by bacteriologic studies or by autopsy. In addition, in 4 other cases there was bronchopneumonia with bloody sputum, of which bacteriologic studies were not made, so that proof of the metastatic origin of the pulmonary disease was lacking.

Smith and Little [15] did some experiments on the pathogenic action of filtrates of cultures of B. coli. The strains were obtained from calves with a choleriform disease (scours), cultures having been made of material from the ileum. A living culture injected intravenously into a calf killed the animal in one hour; the lungs showed marked congestion and some hemorrhages. Intravenous injections of the filtrates of cultures of B. coli were highly toxic for calves. One calf, which was put to death two days after injection of a filtrate, showed congestion and hemorrhages of the lungs; the alveoli contained blood, fibrin and small numbers of polymorphonuclear leukocytes enmeshed in coagulum. Two other calves, which died in five and one-half and twenty-three hours, respectively, showed congestion and hemorrhages of the lungs with coagulation of blood in alveoli.

Charlton [16] and Helmholz and Beeler [17] gave numerous rabbits intravenous injections of B. coli and obtained various focal lesions, but in no instance did they find a pulmonary lesion.

Andreoli [18] presented a clinical study of the role of B. coli and the enterococcus in pulmonary lesions. He believed that under certain influences these organisms could become pathogenic, quit their habitat and penetrate into the circulation and that, further, they could localize in the lungs (as well as in other organs), producing pulmonary lesions of varying severity.

Ilfeld [19] described 3 fatal cases of B. coli septicemia following gastric operation. Two of the patients were examined post mortem, and both showed bronchopneumonia. No cultures of the lungs are mentioned.

Meltzer [20] presented the case record of a 9 day old infant who died from B. coli infection. The infant had bronchitis, pneumonia, meningitis and peritonitis —all caused by B. coli. The pus from the meninges gave a pure culture of B. coli. The lungs showed confluent hemorrhagic bronchopneumonia. The alveoli contained edema fluid, blood and polymorphonuclear leukocytes. There were several abscesses and necrotic areas. Meltzer also described the presence of blue amorphous bodies and meconium within the lung. Bacterial stains on sections of the lungs showed many gram-negative bacilli in bronchi, alveoli and thrombosed veins; clusters of cocci were also seen, but these were few and apparently limited to abscesses in the lungs.

REPORT OF A CASE

The patient was a 47 year old white woman with chronic glomerulonephritis. One month prior to death she had a severe epistaxis and, because of marked anemia, received a transfusion of 500 cc. of compatible blood. Following this, renal failure developed, accompanied by acute bilateral parotitis. Her condition worsened rapidly, and she died in uremia.

The postmortem examination was done about one and one-half hours after death. The left lung weighed 570 Gm. and the right lung 380 Gm. The bronchi contained frothy white

14. Felty, A. R., and Keefer, C. S.: J. A. M. A. **82**:1430 (May 3) 1924.
15. Smith, T., and Little, R. B.: J. Exper. Med. **46**:123, 1927.
16. Charlton, G. A.: J. M. Research **11**:507, 1904.
17. Helmholz, H. F., and Beeler, C.: Am. J. Dis. Child. **14**:5, 1917.
18. Andreoli, G.: Arch. d. mal. de l'app. digestif **19**:165, 1929.
19. Ilfeld, F. W.: Arch. Surg. **31**:632, 1935.
20. Meltzer, J.: Geburtsh. u. Frauenh. **1**:718, 1939.

fluid. Both lungs, especially the left, appeared edematous and congested. The lower lobe of the left lung and the middle lobe of the right lung were heavy and sank in water; they showed considerable loss of crepitation, but no areas of consolidation were made out. Material was taken from the lower lobe of the left lung and from the spleen for culture. This was done by searing the surface of the organ with a red-hot spatula and removing a wedge-shaped piece of tissue with sterile instruments. The specimen of lung yielded a very heavy growth of B. coli and a light growth of an alpha hemolytic streptococcus. The specimen of spleen yielded a light growth of B. coli. The bacillus obtained was gram negative; it fermented lactose and dextrose with production of acid and gas. Unfortunately, the cultures were discarded at this point, before the sections were examined microscopically.

Microscopic examination of the lungs showed bronchopneumonia of the lower lobe of the left lung and the middle lobe of the right lung. The alveoli were filled with an exudate consisting almost entirely of fibrin and polymorphonuclear leukocytes, although small numbers of erythrocytes and macrophages were also present. In the routine sections stained with hematoxylin and eosin, innumerable bacilli were seen lying free in the exudate as well as engulfed in the cytoplasm of macrophages. Sections of the same blocks stained with MacCallum's bacterial stain[21] showed these bacilli to be gram negative. Rare gram-positive cocci were also seen.

Because of the brief interval elapsing between death and the autopsy, because of the heavy growth of B. coli on culture of tissue from a lung and because of the tremendous numbers of gram-negative rods (present intracellularly as well as extracellularly) in the exudate, we considered that in all probability the etiologic agent was B. coli. The possibility of a virus, a toxin or a chemical being the etiologic agent was excluded on the basis of the type of anatomic reaction.[22]

The anatomic diagnosis was: chronic glomerulonephritis; slight hypertrophy of the heart; uremia; anasarca; bronchopneumonia (B. coli); acute parotitis, bilateral; acute ulcerative glossitis (following bites during convulsion); focal hemorrhages of the skin, the liver and the mucosa of the colon; old thrombosis of the right renal vein; thrombosis of the uterine veins; thrombosis of the left internal iliac artery; mural thrombus in the right auricular appendage of the heart.

EXPERIMENTAL STUDY

The purpose of the experiments was to study the effects of living and dead B. coli, as well as of a lysate of B. coli, on the lungs of rabbits.

Materials and Methods.—The animals used in the experiments were young adult rabbits.

The B. coli strains used were cultivated on Douglas agar–blood slants. Cultures of lungs were streaked on Douglas agar–blood plates. The identification of the organisms was made as follows: In each instance, smears were stained with Gram's stain, cultures were planted in lactose as well as in sucrose fermentation tubes, and the Voges-Proskauer and methyl red tests were done. These procedures were carried out with the organisms that were injected into the animals as well as with those that were recovered from the lungs of the animals at autopsy.

For the rabbits given live organisms, stock strains of B. coli communis and B. coli communior were used, as well as a strain of B. coli communior isolated from the sputum of 1 of the 2 patients previously mentioned. This strain was designated B. coli communior (P). The organisms were grown on Douglas agar–blood slants for forty-eight hours and were then suspended in sterile physiologic solution of sodium chloride. Each animal received the contents of three slants suspended in 2 cc. of the saline solution—about 50 to 100 billion organisms.

One group of animals received a similar number of heat-killed organisms. Forty-eight hour cultures of B. coli communior (stock) were suspended in sterile physiologic solution of sodium chloride and kept in a water bath at 63 C. for one hour. Cultures of these suspensions were negative. Each animal received the contents of three slants suspended in 2 cc. of the saline solution.

A group of animals received a toxic extract of B. coli communior (stock) which was prepared by repeated freezing and thawing. The contents of twelve slants (forty-eight hour

21. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 274.

22. Sprunt, D. H.: South. M. J. **31**:362, 1938. Sprunt, D. H., and Camalier, W., Jr.: Arch. Path. **34**:801, 1942.

cultures) were suspended in 9 cc. of sterile distilled water. The suspension was frozen and thawed six times. This failed to kill all the bacteria. The suspension was then filtered through a Seitz filter, and 5.5 cc. of a clear colorless filtrate was obtained. The volume of the filtrate was made up to 8 cc. by addition of sterile saline solution. Each rabbit received 2 cc. of this filtrate.

As a control, a group of rabbits received injections of saline washings of the blood agar medium. A small amount of sterile physiologic solution of sodium chloride was washed over twelve slants, and the slants were scraped so that a blood-tinged suspension was obtained. Each rabbit of the control group received 2 cc. of this suspension.

The inoculations were made as follows: After the animal to be inoculated was anesthetized with ether, the fur of the anterior part of the neck was clipped with scissors and the skin swabbed with 50 per cent alcohol. An incision was made anteriorly in the skin and muscle of the neck, and the material was injected intratracheally with needle and syringe. The edges of the incision were then approximated with silk sutures, and iodine was poured onto the surgical wound.

Autopsies were done immediately on those animals that were put to death. Of the 3 animals that died, 2 were examined only a few hours after death and the third about fifteen hours after death. The tissues of the latter were discarded because of autolysis. Three sets of sterile instruments were used to remove each pair of lungs. The skin was covered with iodine, and was then stripped back with one set of instruments. The subcutaneous tissues were covered with iodine, and the anterior part of the thoracic cage was removed with another set of instruments. The heart and the lungs were then removed en masse with the third set of instruments. In most instances a piece of lung was cultured immediately. In some instances a piece of lung was not taken, in order to obtain better inflation of the lungs with air.

After a piece of lung was removed for culture, the lungs were inflated with air and fixed in Helly's solution. After fixation of the lungs, blocks of tissue were taken and embedded in paraffin. Sections were stained with hematoxylin and eosin and with MacCallum's bacterial strain.

Experimental Groups.—In all, 31 rabbits were used. All materials were injected intratracheally, each rabbit receiving a total volume of 2 cc.

Group A (rabbits 1 to 6) received living B. coli communior (P).

Group B (rabbits 7 to 13) received living B. coli communior (stock).

Group C (rabbits 14 to 19) received living B. coli communis (stock).

Group D (rabbits 20 to 23) received heat-killed B. coli communior (stock).

Group E (rabbits 24 to 27) received the lysate prepared by repeated freezing and thawing cultures of B. coli communior (stock).

Group F (rabbits 28 to 31) received sterile saline washings of the blood agar medium.

Except for rabbits 2, 6 and 12, which died after the injections, the rabbits were killed on the fourth day by a blow on the back of the neck. Rabbit 2 died in twenty-four hours, rabbit 6 in forty-eight hours and rabbit 12 in ninety-six hours. Rabbit 12 was discarded because of autolysis.

A summary of the experiments is presented in the accompanying table.

OBSERVATIONS

Groups A, B and C.—The lungs of the rabbits from groups A, B and C presented identical lesions and will be described together.

Gross Changes: All rabbits in these groups showed marked to severe degrees of pneumonia grossly. In each rabbit the great bulk of the lung tissue was involved. There were large irregular areas of consolidation, most marked in the posterior portion of the lungs, near the hilus. These appeared as dull purple areas, which were firm and airless and of rubbery consistency. In many animals there were, in addition, varying areas of hemorrhage and necrosis, brownish red in color, bounded by a pale gray border. In some lungs pronounced edema was also present. In the lungs of a few rabbits there was also a thick pleural exudate. After fixation of the lungs the cut surfaces showed similar abnormalities except that the colors differed from those of the lungs in the unfixed state. Now the dull purple consolidated regions were seen as grayish white firm areas which faded gradually into the more crepitant darker regions of the lungs. The necrotic areas, bounded sharply by a grayish white border, were now paler and stood out in marked contrast to the less inflamed regions

of the lungs, which appeared as spongy dark brown tissue. These gross appearances are illustrated in the photographs of the lungs taken after fixation (figs. 1 to 4).

Microscopic Changes: A pronounced degree of pneumonia was seen in all lungs. Three main types of lesions were seen. The first was an interstitial mononuclear type, characterized by marked thickening of the interstitial tissue of the lungs. This was caused by edema, congestion and marked cellular infiltration. The cellular infiltrate consisted mostly of large and small mononuclear cells, the former predominating; the former appeared to be macrophages and the latter lymphocytes. Moderate numbers of polymorphonuclear leukocytes were also present. The alveoli in many areas contained moderate numbers of large macrophages and polymorphonuclear cells, in equal proportion. Some alveoli also contained edema fluid. There was little or no fibrin. Many multinucleated giant cells were present in some alveoli and appeared to line the alveolar septums in some regions. The lining cells of the alveoli were greatly swollen and often resembled the macrophages in the lumens of the

Summary of Experiments

Rabbit	Material Injected	Fate of Animal	Results of Culture of Tissue from Lungs		Pulmonary Lesion	Organisms Seen in Tissues
			Amount of Growth	Organism		
1	B. coli	Killed*	++++	B. coli	+++	Few
2	communior	Died, 24 hr.	++++	communior	++++	Many
3	(P)	Killed	Culture not made		++++	Few
4		Killed	+		+++	Few
5		Killed	Culture not made		+++	Few
6		Died, 48 hr.	Discarded			
7	B. coli	Killed	+	B. coli	+++	Few
8	communior	Killed	Culture not made	communior	+++	Few
9	(stock)	Killed	+++		+++	None
10		Killed	+++		+++	None
11		Killed	++		+++	None
12		Died, 96 hr.	Culture not made		++++	Many
13		Killed	Culture not made		++	None
14	B. coli	Killed	Culture not made	B. coli	+++	None
15	communis	Killed	++	communis	++++	Rare
16	(stock)	Killed	+		+++	Rare
17		Killed	+		+++	None
18		Killed	Culture not made		+++	None
19		Killed	Culture not made		++	Rare
20	Heat-killed	Killed	None	.....	++	Few
21	B. coli	Killed	None		++	Few
22	communior	Killed	None		+++	Few
23	(stock)	Killed	None		+++	Few
24	Lysate of	Killed	None	.....	++	None
25	B. coli	Killed	None		+	None
26	communior	Killed	None		+	None
27	(stock)	Killed	None		++	None
28	Saline	Killed	None	.....	None	None
29	washings of	Killed	None		None	None
30	medium	Killed	None		None	None
31		Killed	None		None	None

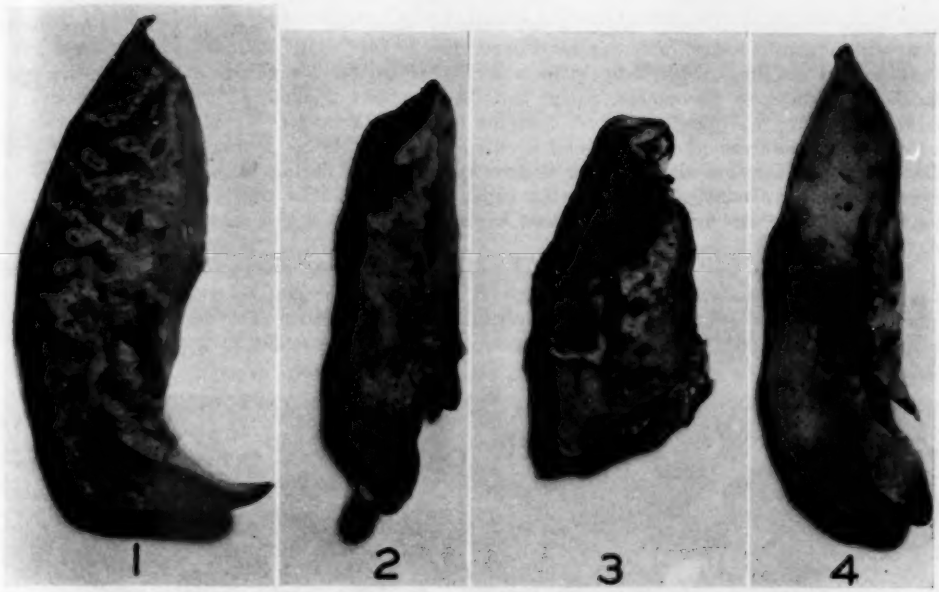
* Killed on the fourth day.

alveoli. The bronchi were loaded with pus and often showed ulceration. The lymphatics were filled with debris, fluid and inflammatory cells. Many blood vessels showed a considerable round cell infiltration of all coats and some intimal fibroblastic proliferation. The pleura in a few cases was covered with a thick fibrinopurulent exudate. This type of lesion is illustrated in figures 5, 6 and 7.

The second type of lesion consisted of infarct-like areas of necrosis (figs. 5 and 8). These regions showed a great deal of karyorrhexis. In many areas the shadowy outlines of the alveolar septums were still visible, and here the alveoli contained large pink-staining structures, apparently swollen macrophages which had been destroyed. In these necrotic areas the blood vessels showed a purulent and necrotizing inflammation accompanied by thrombosis. The areas of necrosis may have been due to a greater concentration of bacteria in these regions.

The third type of lesion consisted of a plugging of alveoli with masses of pink-staining material, which appeared to be conglutinated erythrocytes in various stages of degeneration.

Sections of lung stained with the bacterial stain showed small numbers of gram-negative bacilli; these were seen lying free in the exudate as well as within macrophages. In addi-



Figs. 1 to 4.—Photographs of rabbit lungs fixed in Helly's fluid. The paler areas are the severely inflamed and necrotic portions. The lungs are from rabbit 5 (fig. 1) rabbit 15 (fig. 2), rabbit 3 (fig. 3) and rabbit 4 (fig. 4). The lung of rabbit 3 (fig. 3) shows necrosis and thick pleural exudate. In the lung of rabbit 4 (fig. 4) the grayish white areas are the firm rubbery consolidated parts of the lung.

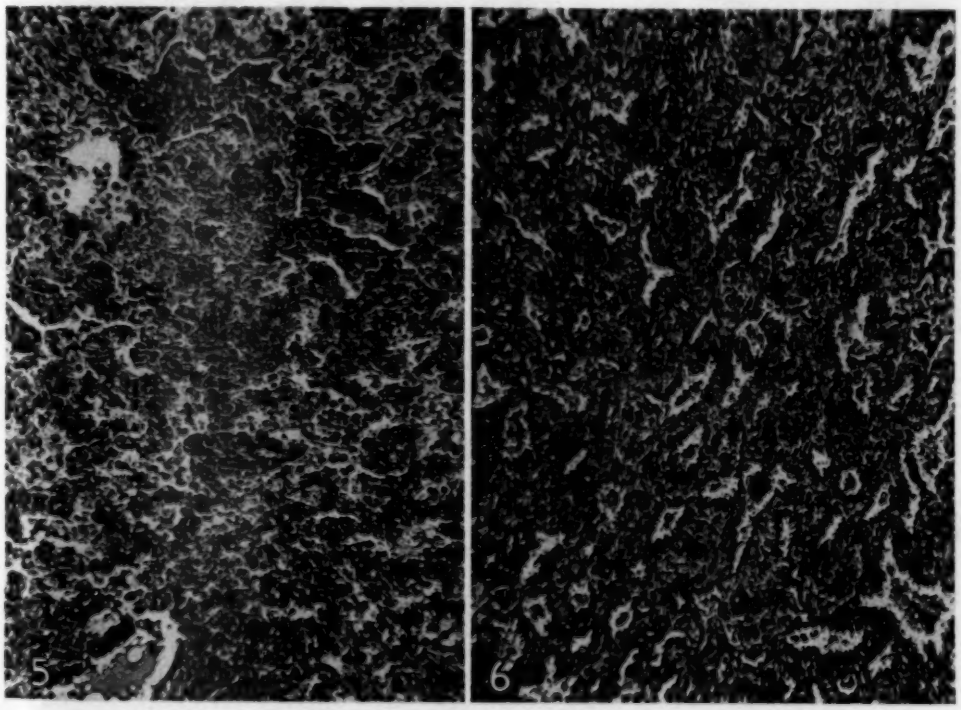


Fig. 5.—Section of an inflated lung of rabbit 20, showing the characteristic lesion. Note the necrotic area in the upper right hand corner (hematoxylin and eosin; × 115).

Fig. 6.—Section of an inflated lung of rabbit 27, showing marked thickening of the interstitial tissues (hematoxylin and eosin; × 115).

tion, large numbers of very short gram-negative rods and coccobacillary structures were seen within macrophages, presumably degenerated forms of B. coli. No other organisms were seen in the lungs. The only lungs which presented large numbers of the gram-negative bacilli were those of the rabbits which had died (rabbits 2 and 12). In these rabbits the greatest numbers of organisms were seen in the necrotic areas. It is possible to account for the large numbers of bacilli found in these two animals by postmortem proliferation of the organisms. On the other hand, it is just as likely that during life the bodily defenses of these rabbits were unable to prevent the rapid multiplication of the bacilli, which multipled quickly and killed the animals; this could explain the death of the animals and the presence of numerous bacteria in the lungs.

In groups A, B and C, the lungs were cultured in half the cases; in all of these B. coli was recovered in pure culture. In groups A and B the organism recovered was B. coli communior, and in group C, B. coli communis. Thus, in each case the type of organism recovered corresponded to the one that was originally inoculated.

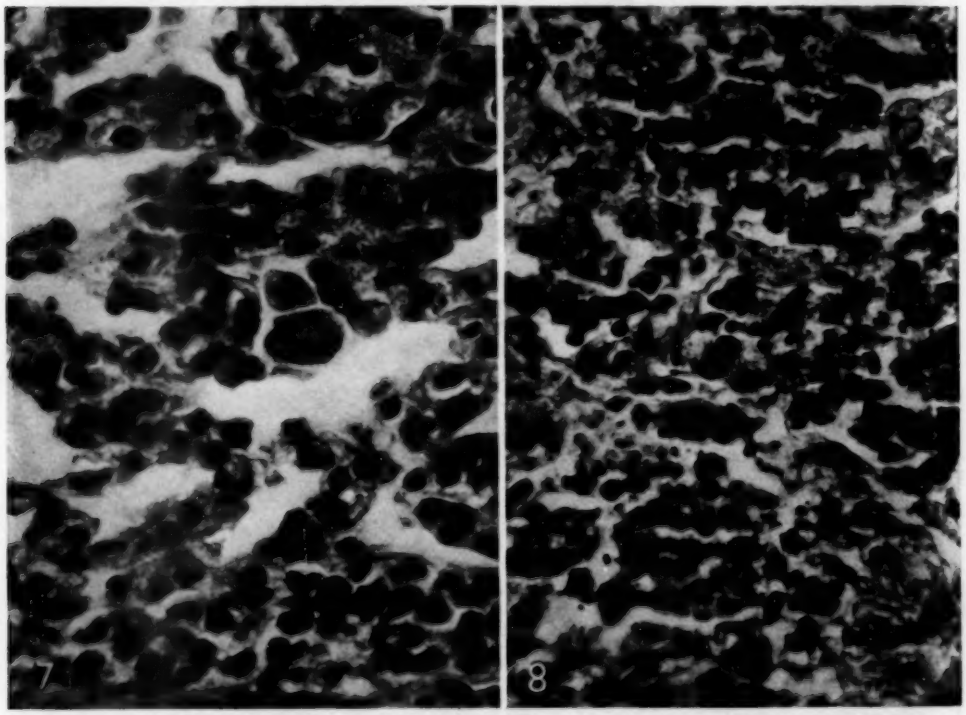


Fig. 7.—Section of an inflated lung of rabbit 7. Note the marked infiltration of the interstitial tissues by mononuclear cells, as well as the macrophages in the alveoli (hematoxylin and eosin; × 687).

Fig. 8.—Section of an inflated lung of rabbit 20, showing an area of necrosis. Note the karyorrhexis and the swelling of the lining cells of the alveoli (hematoxylin and eosin; × 687).

Group D.—The rabbits which received the dead organisms had essentially the same types of pulmonary lesions as the rabbits which received the live organisms except that generally the lesions were less severe. The lesions were moderate in 2 animals and moderate to marked in the other 2.

Sections stained with the bacterial stain were identical in appearance with those of groups A, B and C. Few gram-negative bacilli and numerous degenerated forms, both intracellular and extracellular, were seen. No other organisms were seen.

Cultures of tissues from the lungs of all 4 rabbits were negative.

Group E.—The rabbits which received the lysate also had lesions essentially similar to those in the previous groups but much less marked in degree and extent. Only a small

portion of the total pulmonary tissue was involved in each rabbit. In 2 rabbits the lesion was slight, and in the other 2, moderate. Focal necrosis was present in only 1 animal. The chief response was marked interstitial thickening, caused by edema, congestion and mononuclear cell infiltration (fig. 6).

No organisms were seen in the tissues. Cultures of material from the lungs of all 4 rabbits were negative.

Group F.—The lungs of the animals which received the saline washings of the medium revealed no lesions except slight congestion and slight leukostasis of the capillaries. No organisms were seen in the tissues. Cultures of material from the lungs of all 4 rabbits were negative.

COMMENT

After the information obtained from the experiments was put together with that from the case of pneumonia studied at autopsy, it was concluded that B. coli can cause pneumonia in man. The fact that pulmonary lesions were obtained with dead organisms and a lysate of the organisms as well as with live organisms seems to point to the action of a toxin of B. coli as one of the etiologic factors. There was a difference between the type of reaction in the human lung and that in the rabbit lung. The former showed fibrinopurulent exudation into the alveoli, while the latter showed interstitial mononuclear pneumonia with focal necrosis.

Apparently, pneumonia caused by B. coli is uncommon in man, but we suspect that many cases have been missed because of the general tendency to attach little or no importance to the presence of this organism in laboratory specimens, especially those obtained at autopsy. This is particularly true of specimens obtained from the lungs because, although the importance of the role of B. coli in infections of the genitourinary tract and the gastrointestinal system is generally appreciated, there is little information in the literature about the effect of B. coli on the lungs. There is no doubt that in most instances the isolation of B. coli from autopsy material signifies contamination or, perhaps, postmortem invasion of tissues by the organism; still, the consideration that the presence of B. coli in these specimens may be of some significance may result in finding more pulmonary lesions caused by this organism.

The question arises as to how the organism reaches the lungs and from what sources.

Kreibich [1] stated that an infection of the lungs with B. coli as a result of aspiration cannot be excluded, that at least it seemed probable in a case of carcinoma or of a diverticulum of the esophagus with break-through into the trachea or in a case in which vomited material was aspirated. He thought, however, that in most cases B. coli pneumonia was caused by hematogenous infection, either from the bowel or from inflammatory processes of the urogenital tract.

Felty and Keefer [14] in their discussion of B. coli septicemia listed the portals of entry of the organism into the blood stream in their series of 28 cases. The portal of entry was the urinary tract in 16 cases, the female genital tract in 6, the intestinal tract in 2 and a wound infection in 1; in 3 cases it was undetermined. They quoted the results of Jacob's [23] studies as follows:

> In Jacob's compiled cases, the biliary passages were most frequently found as the primary focus; after this in order of frequency were the urinary tract, the intestine and the female genital tract. Cases of B coli sepsis in which the intestine was regarded as the portal of entry either followed acute inflammatory processes of the intestine (typhoid, dysentery, etc.) or developed subsequent to appendix abscesses with thrombophlebitis or peritonitis.

Andreoli [18] expressed the belief that the gastrointestinal tract was often the source of pulmonary infections and that the infecting organisms reached the lungs

23. Jacob, L.: Deutsches Arch. f. klin. Med. **97**:303, 1909.

through the general circulation. He quoted the experiments of Moscati and of Binet and Loubry,[24] who demonstrated in the dog a connection by way of lymphatics between the abdominal cavity and the lungs. Rouviere,[25] however, referring to these experiments, stated that the anatomic disposition which assures this manner of communication in the dog does not exist in man.

Ilfeld,[19] in discussing fatal cases of B. coli septicemia following gastric operations, stated:

> . . . It seems likely that the organisms were in the stomach at the time of operation and were thus introduced into the blood stream. It is well known that the stomach may contain B. coli.

He then quoted several authors who had reported finding this organism in the gastric juice.

Since B. coli may be present in gastric juice in some instances, it seems fairly reasonable to assume that aspiration of the contents of the stomach may introduce these organisms into the lungs.

To summarize, it seems that B. coli can reach the lungs by being aspirated and by being carried there in the blood stream. In regard to man it seems unlikely that the organisms can reach the lungs from the gastrointestinal tract by means of direct lymphatic channels.

In our human case of B. coli pneumonia, aspiration may have been the method of entry of the bacteria into the lungs. The patient was comatose, and aspiration of the contents of the nasopharynx or of the stomach under such circumstances is quite common, if not the rule. We cannot be certain of the source of the organisms, but we do know that the patient had acute ulcerative glossitis following biting of the tongue during convulsions. Sections of the tongue stained with MacCallum's bacterial stain showed many gram-positive cocci and bacilli and many gram-negative bacilli. It may be that the B. coli pneumonia resulted from aspiration of the organisms which may have been present in the mouth. Sections of the parotid gland stained with the bacterial stain showed only gram-positive cocci.

SUMMARY AND CONCLUSIONS

The literature on pneumonia caused by B. coli is scanty and, for the most part, inadequate and inconclusive. A case of pneumonia in man studied at autopsy is presented, in which it seemed reasonably certain that the etiologic agent was B. coli. The response was a fibrinopurulent exudate filling the alveoli and bronchi. This type of reaction excluded the possibility that a virus, a toxin or a chemical was the etiologic agent.

Intratracheal injections of live cultures of B. coli into rabbits produced interstitial mononuclear pneumonia and focal areas of necrosis. Similar lesions, although of lesser severity, were produced by using heat-killed cultures of B. coli and a lysate obtained from cultures of B. coli. Thus it seemed that a toxin of B. coli was one of the etiologic factors in the production of the lesions in the rabbits.

It is concluded that B. coli can produce pneumonia in man and in rabbits.

A difference is noted between the response in the human being—fibrinopurulent pneumonia—and the response in the animals—interstitial mononuclear pneumonia.

24. Binet, L., and Loubry, J.: Bull. Acad. de méd., Paris **94**:1276, 1925.

25. Rouviere, H.: Anatomie des lymphatiques de l'homme, Paris, Masson & Cie, 1932, p. 218.

CONGENITAL ANEURYSMS OF THE CEREBRAL ARTERIES

AN EMBRYOLOGIC STUDY

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The occurrence of actually congenital aneurysms of the arteries at the base of the brain has often been doubted, many writers considering all aneurysms essentially pathologic. The present paper seeks to answer the question by a study of the development and growth of the arteries of this system and by a detailed examination of these vessels in subjects of various ages, from embryo to adult. In the literature there are scattered drawings of the cranial arteries, included in numerous general descriptions of particular embryos, and Mall [1] made a special study of the early cerebral system, but nowhere, to my knowledge, have the developmental changes been consecutively followed through prenatal life and compared critically with the conditions present at birth and in later years. For this reason, and also because these changes were found to bear so directly on the subject, it seemed necessary to include such a study here.

The growth of the brain and of the cranial arteries can be followed in the series of embryos shown in figures 1 to 5. The first two of these are reconstructions of pig embryos, since no suitable human material of these younger stages was available, but there is no reason to suppose that any essential species difference is present at these early stages. The other figures represent the conditions found in human embryos from the Harvard Embryological Collection. At 5 mm. the brain already exhibits the three primary vesicles, forebrain, midbrain and hindbrain, and is bent so that the morphologic tip, carrying the optic vesicles, points caudally. The earliest branch of the aortic arch forms the trunk of the future internal carotid artery and, growing forward, encounters the optic vesicle first, sends branches above and below it and continues along the under side of the brain, following its lesser curvature to the base of the hindbrain, where it is lost in a net of capillaries. At this age all the cerebral arteries belong to the carotid system, since there is no connection with the future vertebral vessels.

From the Harvard University Medical School.

1. Mall, F. P.: Am. J. Anat. **4**:1, 1904.

Distal to the vessels to the eye, numerous capillaries spring at right angles from the trunk toward the brain. The arrangement is bilateral, with no connection between the two sides.

In the embryo of 7 mm. the subdivisions of the brain are more definitely marked, especially in the forebrain, where the optic vesicle has become stalked (and is represented as cut through this narrower portion) and displaced ventrally by the growth of the hemisphere. Together these two structures occupy the tip of the forebrain or telencephalon; the rest of the forebrain is known as the diencephalon and is destined to produce the thalamus and subthalamic structures. The capillaries to the brain have lengthened and by union of branches have formed a close-meshed rectangular net, with some members running parallel to the trunk. The first branch of the arterial trunk, below the optic stalk, remains small. The second branch, arising from a network, divides further, one branch running behind the swelling of the hemisphere, one to the middle of it and one to its ventral side. They may be recognized respectively as the anterior choroid, middle cerebral and anterior cerebral arteries. The last continues on toward the nasal organ, now represented as a shallow pit of surface epithelium on the ventrolateral tip of the head. The anterior cerebral artery is thus shown to be primarily the artery of the olfactory organ, though later this connection is represented only by a minor branch. The posterior cerebral and superior cerebellar arteries are recognizable as two ill defined groups of peripheral branches arising from a nearly continuous net. As shown by Evans,[2] whose figures agree closely with mine, a full injection of the vessels of the head reveals that the capillary net is continued into veins lying peripheral to the arteries. All the early branches run along the side of the brain without piercing the brain substance.

The human embryo of 17.8 mm., probable age 7 weeks (fig. 3), has been fully reconstructed by Thyng[3] and is still available for a further detailed study of the cerebral arteries. The hemisphere extends beyond the limit of the median forebrain rostrally and overlaps it caudally. The optic stalk is relatively smaller and more ventrally placed. A former minor branch of the anterior cerebral artery has become the main trunk and extends with a sharp curve dorsally over the end wall of the median forebrain, the lamina terminalis, under cover of the overhanging hemisphere, to supply the choroid plexuses of both the third ventricle and the lateral ventricle where they meet rostrally

2. Evans, H. M.: Development of the Vascular System, in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Co., 1912, vol. 2, pp. 570-709.

3. Thyng, F. W.: Am. J. Anat. **17**:31, 1914.

above the foramen of Monro. At the ventral side of the hemisphere, where the two anterior cerebral arteries, right and left, make their sharp turns dorsally, they run close to each other and give off several sprouts, which anastomose to form an intricate plexus, the future anterior communicating artery. The middle cerebral artery has become more definite, its branches spreading over the lateral surface of the hemisphere. The numerous vessels previously recognized as a group representing the posterior cerebral artery are now in the process of becoming branches of a single trunk by the absorption of all but one of the roots of the capillary plexus from which they formerly arose. The branches cover the lateral wall of the diencephalon and also of the midbrain. One branch runs forward under the caudal pole of the hemisphere to supply the choroid plexus of the third ventricle from behind. The corresponding vessel on the opposite side sprouts directly from the main trunk. Mall called a closely similar vessel the anterior choroid artery, which it almost certainly is not, for the latter is represented more rostrally, running partly under cover of the hemisphere, arising from what can now be recognized as the future continuation of the internal carotid artery. The vessel in question is more properly a primitive posterior choroid artery, later replaced by another more peripheral branch of the posterior cerebral artery. The two lateral basilar arteries have joined with the vertebral arteries and have fused along the base of the hindbrain to a point between the roots of the superior cerebellar artery (which has also acquired a single stem) and the posterior cerebral trunk. From all these various arteries short capillary branches, usually arising at right angles, now penetrate the brain substance.

From this point the history of the cerebral arterial pattern, the first steps of which are evident in an embryo of 45 mm. (fig. 5), is governed by the further growth of the hemispheres, the development of the basal ganglions and of the ventral nuclei of the midbrain, and by the addition of the large fiber tracts. The thalamus represents the growth of many nerve cells in the dorsolateral wall of the diencephalon, causing the brain wall to thicken and bulge laterally. The caudate and lenticular nuclei develop in the floor of the hemisphere, the caudate bordering the ventricular cavity, the lenticular in a more ventral and lateral position. As the hemisphere assumes its older curved form, the head of the caudate nucleus bulges forward beyond the foramen of Monro, while the body arches backward over the lenticular nucleus. The nerve fibers comprising the internal capsule course between the two and continue downward peripheral to the thalamus, where they are originally surface fibers, even as in the peduncles, but are soon covered in by the fusion, from the foramen of Monro backward, of the lateral wall of the forebrain with the mesial wall of the overhanging hemisphere. This progressive

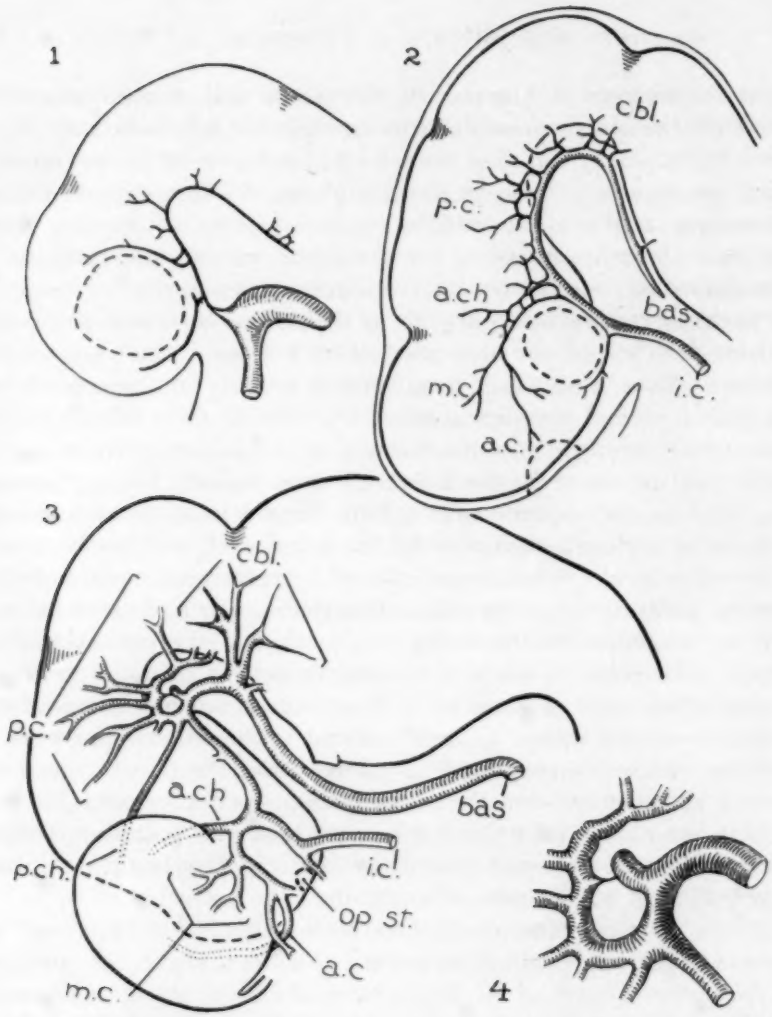

Fig. 1.—Arterial supply to the head of a pig embryo of 5 mm. The positions of the forebrain, with optic vesicle, the midbrain and the hindbrain are indicated. Note the cerebral plexus from the main trunk arising from the aortic arch.

ABBREVIATIONS

a.c., anterior cerebral artery	*i.c.*, internal carotid artery
a.ch., anterior choroid artery	*m.c.*, middle cerebral artery
bas., basilar artery	*op.st.*, optic stalk
cbl., superior cerebellar artery	*p.c.*, posterior cerebral artery
ch., optic chiasm	*p.ch.*, posterior choroid artery
c.pl., commissural plate	*p.com.*, posterior communicating artery

Fig. 2.—Arterial supply to the brain of a pig embryo of 7 mm. The hemisphere is appearing above the optic vesicle. The individual cerebral arteries are represented by groups of plexuses. The anterior cerebral artery runs to the nasal pit. Further explanation is given in the text.

Fig. 3.—Brain and cerebral arteries of a human embryo of 17.8 mm.

Fig. 4.—Enlargement of a portion of figure 3, showing formation of a single stem from a plexus representing the posterior cerebral artery.

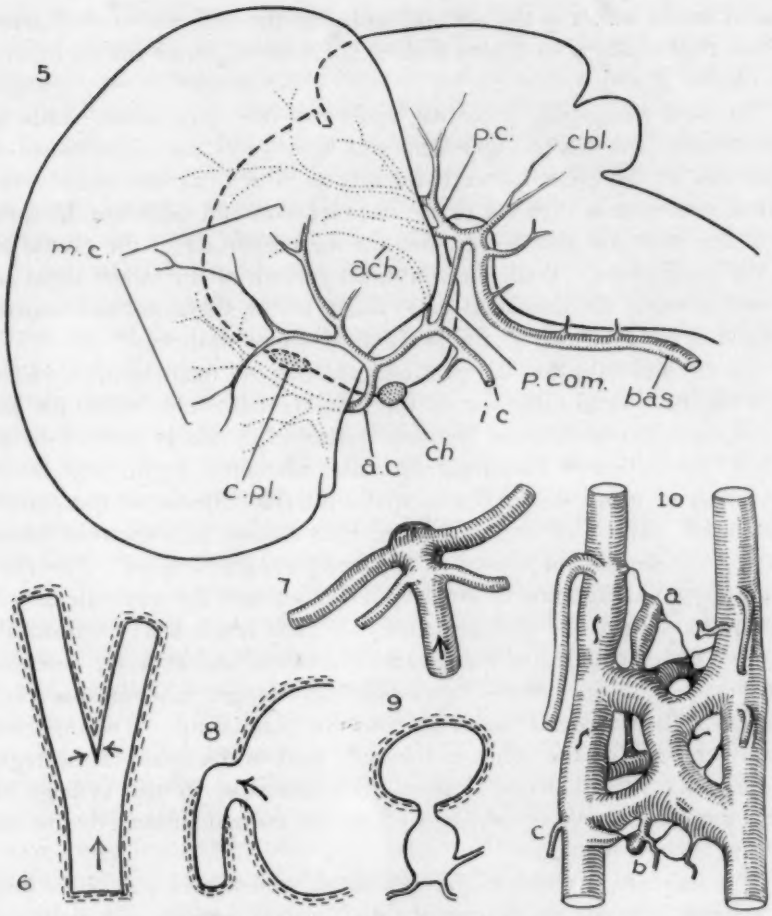

Fig. 5.—Cerebral arteries of a human embryo of 45 mm. at the beginning of the third month.

Fig. 6.—Diagram to show the probable direction of pressure at the forks of an acute bifurcation, with effect on muscle formation.

Fig. 7.—Terminal bifurcation of the basilar artery of a human embryo of 42 mm., with an aneurysmal pouch between the widespread posterior cerebral arteries.

Fig. 8.—The case of an artery of Heubner's system arising from the anterior cerebral artery in a woman of 59 years. Note the wide muscular gap with shallow pouch. See text for further comment.

Fig 9.—Minute aneurysm from the side of the anterior cerebral artery of a human embryo of 45 mm., resulting from irregular degeneration of certain members of a periarterial capillary plexus.

Fig. 10.—Anterior communicating artery of an infant 1 week old, showing complicated plexus formation, with closure of some portions, and two aneurysmal pouches (*a* and *b*) with little or no outlet. An artery of Heubner's system is shown (*c*).

fusion forces before it the pia and with this the anterior choroid artery, which thus changes its course and its angle of origin, as can be followed in figures 3 and 5.

Beyond the point of actual fusion of the two brain walls the membranes are closely apposed, and this gives an opportunity for branches of the posterior cerebral artery, which, as has already been noted, is primarily directed to the diencephalon and midbrain, to sprout in a new direction and supply also the mesial surface of the caudal half of the hemisphere. With the continued growth of the latter, these new vessels assume the dominant role; those to the thalamus and superior colliculus are relegated to the position of minor branches.

In the midbrain the roof produces the corpora quadrigemina (whose bulk has no special influence on the basal vessels) and, below the level of the aqueduct of Sylvius, becomes thickened by the growth of the red nuclei, the reticular formation and the substantia nigra, and by the extension of many of the fibers of the internal capsule as the cerebral peduncles. Much of the thickening thus caused is somewhat lateral, leaving the depression known as the interpeduncular space. The result is the partial obliteration of the deep notch between the forebrain and the hindbrain, seen in the younger embryos. The trunk artery follows this change, and the stems of the posterior cerebral and superior cerebellar arteries of necessity become correspondingly longer without appreciable change in their angle of diversion from the main trunk. The numerous small vessels from the trunk to the basal part of the brain in the region of the notch may, however, suffer much distortion by this change, and even the main trunk vessel, deprived of its normal linear growth, may tend to become tortuous.

The increase in width of the individual hemispheres and of the brain as a whole changes the course of other cranial arteries and, with this, the angle at which they leave the parent stem. The middle cerebral artery runs to the lateral surface of the hemisphere, the anterior artery to the mesial surface. Since their point of origin from the internal carotid artery lies close to the base of the brain, growth in width of the hemisphere spreads them apart until at birth the angle between them is almost 180 degrees. The gradual spreading of this angle can be followed in figures 1 to 5. In the youngest embryo the angle is acute, about 20 to 25 degrees as measured in the sections; at 45 mm. it is over 90 degrees. The two internal carotid arteries and the two posterior communicating arteries are merely carried bodily laterally, but the angle at the junction of the latter two with the single median basilar artery is much increased. This junction takes place topographically, as has been noted, between the origins of the embryonic posterior cerebral and superior cerebellar arteries; in the adult the small section of the original

trunk vessel from the junction to the original root of the posterior cerebral vessel is considered as a portion of the latter artery, the posterior communicating artery becoming a branch. This is due to the increased blood supply from the vertebral arteries after their fusion with the basilar artery, which causes a reversal of flow in the latter and makes the posterior cerebral arteries appear as its terminal bifurcation. The angle between them at their divergence may be as much as 180 degrees in the adult, though in the original pattern the angle between the two arterial trunks was very narrow. Since many cerebral aneurysms occur at the angles between the anterior and middle cerebral arteries and at the terminal bifurcation of the basilar artery, the changes due to growth at these two points are especially significant. The superior cerebellar arteries, since they sprout from the two sides of the median basilar artery, merely change their position in the wall of the parent vessel to accommodate the increasing width of the hindbrain, without essentially altering their angle of emergence.

Another structure whose growth influences the course of one of the main basal vessels is the corpus callosum. This develops, as was shown by Marchand,[4] in the commissural plate, a thickening in the lamina terminalis or end plate of the median forebrain, shown in figure 5. Through this thickening fibers grow from one hemisphere to the other in two bundles, forming the anterior commissure below, the corpus callosum above. The increasing number of fibers in the latter causes the plate to bulge forward as the genu of the corpus callosum, first recognizable in the fourth month. The originally terminal branch of the anterior cerebral artery, ending in the choroid plexuses, is thus pulled away from this connection and is diverted around the genu to the dorsum of the corpus callosum, where it is in position to send further sprouts to the medial wall of the hemisphere above. As the frontal lobe increases in size, other branches supply the rest of its medial surface, and as the genu enlarges, it pulls the artery bodily forward with it.

The primary and secondary branches of all of the cerebral arteries are submitted to a peculiar type of disturbance. The enlargement of the hemisphere is not accomplished by addition to the two ends of the structure, such as one is accustomed to find, for instance, in the growth of the long bones, but by interstitial growth due to universal increase in the number of nerve cells and fibers. The growth of the hemisphere is most rapid in the early months of fetal life and is retarded later, when the appearance of the convolutions allows the cortex to continue its expansion without adding proportionately to the dimensions of the hemisphere. Still later the bulk is again increased by the progressive myelination of the nerve fibers. The middle and posterior cerebral

4. Marchand, F.: Arch. f. mikr. Anat. **37**:333, 1891.

arteries both approach the hemisphere from the center of its lesser curvature, directed at right angles to its length, their branches spreading fanwise. As interstitial growth proceeds, the branches are spread farther apart. The tendency is noticeable in figures 2, 3 and 5. At birth these angles often approach 180 degrees.

The many small branches that arise from the arteries of the circle of Willis to feed the basal ganglions and subthalamic regions can usually accommodate themselves by lengthening and coiling to any change in position of the stem vessels. The small branches of the anterior cerebral artery to the head of the caudate nucleus are, however, an exception. This artery has been pulled so far forward by the growth of the corpus callosum and the frontal lobe that the anterior perforated space through which the branches entered the brain is left far behind, and the direction of the branches, which originally ran at right angles to the trunk or even pointed forward, is reversed. This effect is most noticeable in the more anterior branches. As a group these branches were described by Heubner [5] in 1872 and are known as Heubner's system; they have been carefully drawn by Aitken [6] and can be recognized in figure 10. They offer another example of the spreading of the angle at arterial forks.

All the bifurcations described as showing angles expanding as the result of the growth of the brain are frequent sites of aneurysms. Turnbull [7] suggested that there is an inherent weakness at these points, and Forbus [8] found at many bifurcations a "medial defect," a small area in the fork of the bifurcation devoid of any muscular coat, though intima, elastica and adventitia remain intact. Such deficient areas were quite frequent not only in the cerebral arteries, whether aneurysms were present or not, but also in the coronary and mesenteric vessels, always at acute angles. Forbus postulated that the internal apex of a bifurcation is the point of greatest pressure from the force of the blood stream and that this occasionally ultimately results in eversion of the unguarded apical area in the form of an aneurysm. He sought to demonstrate his point by the use of glass models so made that a small tube opened into the apex of a fork of a larger tube, continuing the direction of the main trunk, and found that sudden increases of pressure were recorded as greater within the central tube than at any other point in the apparatus. The usual absence of the elastic lamina in aneurysms he attributed to degeneration due to constant stretching of the unsupported tissue.

Later writers did not approve this explanation. Tuthill [9] considered the "medial defects" as embedding artefacts, the widening of slits

5. Heubner, O.: Centralbl. f. d. med. Wissensch. **10**:817, 1872.
6. Aitken, H. F.: Boston M. & S. J. (supp.) **160**:1, 1909.
7. Turnbull, H. M.: Quart. J. Med. **8**:201, 1915.
8. Forbus, W. D.: Bull. Johns Hopkins Hosp. **47**:239, 1930.
9. Tuthill, C. R.: Arch. Path. **16**:630, 1933.

between adjacent muscle bundles, the fibers of which may run in different directions and pull apart with shrinkage; Strauss and co-workers [10] substantiated the observations, but the rather common occurrence of the defects as compared with the infrequency of aneurysms "leads us to question Forbus' belief that they are congenital anomalies." These authors thought most cerebral aneurysms due to arteriosclerosis. Glynn [11] also found the "defects" in a large percentage of cases out of all proportion to the few aneurysms encountered, and sought to show that the muscle layer is unimportant in maintaining the strength of a vessel wall since the elastic layer alone, with most of the media scraped away, will withstand pressures much higher than those of hypertension. Richardson and Hyland [12] also found the "defects" but agreed that while they may predispose to and determine the sites of aneurysms there must be a superadded lesion which acts by weakening the elastica locally.

Forbus suggested as the cause of the medial defects that arterial branches may form their own independent coats and that there may be a failure of the two muscular systems to unite, though he added that this theory does not adequately explain why the defect should always be located at the acute angle. Study of many acute bifurcations in embryonic arteries at the period when their muscular coats are being differentiated suggests a different initial cause for the gaps. It is known that certain vessels, such as the cranial veins, which rest immediately against the unyielding surface of the skull, are devoid of most or all of their muscular coat on the adjacent side. Also it has been shown that on the embryonic left pulmonary aortic arch, the future ductus arteriosus, the musculature fails to develop where the vessel is partially encircled by the recurrent laryngeal nerve (Bremer [13]). Apparently the muscularis develops only when the intima requires additional strengthening to withstand the increasing centrifugal force of pulsation; any support will serve. At the acute fork of a bifurcation, at an angle of 20 degrees or so, if the edge at the internal apex is sufficiently sharp, the force of the stream will be readily deflected at the apex, and along the divergent sides will be resolved into two components, one continuing its original course, the other directed centrally, as is shown by the arrows in figure 6. The two adjacent walls will be pressed against each other and will support each other; no musculature will develop until the branches become so far separated that the elasticity of the intima will no longer allow the two walls to touch. From this point onward the

10. Strauss, I.; Globus, H. H., and Ginsburg, S. W.: Arch. Neurol. & Psychiat. **27**:1080, 1932.

11. Glynn, L. E.: J. Path. & Bact. **51**:213, 1940.

12. Richardson, J. C., and Hyland, H. H.: Medicine **20**:1, 1941.

13. Bremer, J. L.: Anat. Rec. **27**:1, 1924.

strengthening musculature is necessary. Sometimes the muscle develops further toward the apex on one branch than on the other, or on a main trunk than on a branch, the naked intimal wall then being supported by the outer surface of the other muscular sheet. An example of such asymmetry is shown at the acute angle of the branch in figure 8. The principle is illustrated at the sharp bow of a rapidly moving boat; the water meeting the rigid slanting surface forms a wave directed laterally. With a blunt bow, on the other hand, like that of a square-bowed scow, the whole force of the water is deflected in a wave thrown directly forward. In the apparatus used by Forbus the apex is broadened by the very insertion of the median tube, which thus feels the whole impact of the center of the stream. His results, therefore, apply to blunt or "tuning fork" angles, which in the embryo do not show the "medial defects."

In the subsequent spreading of a bifurcation or fork in which a "defect" is already present, the wall may remain naked or be strengthened by new growth of muscle from the sides. The latter is by far the more common result. The type of reaction may possibly be decided by the rapidity of the spreading. Two instances of continued absence of muscular support are shown in figures 7 and 8. The former depicts the condition in a human embryo of 42 mm., the beginning of the third month, at the bifurcation of the basilar artery into the two posterior cerebral arteries. The roots of the superior cerebellar arteries are also shown. Opposite the end of the basilar artery, in the axis of the blood stream, is a small dome-shaped swelling, very thin walled as compared with the main vessels. This is almost certainly an aneurysm. At another range of life, in a woman of 59 years, the condition shown in figure 8 was found. This depicts the junction of an artery of Heubner's system with the anterior cerebral artery, as at *c* in figure 10. The branch has completely reversed its direction. One may interpret the conditions found by supposing that the upper (anterior) gap in the musculature developed when the angle was acute forward, that with reversal this portion stretched without developing musculature and that this occurred early enough to allow the formation of a second "defect" when the posterior angle became acute. The point to be stressed at present is that the original gap has remained throughout life unsupported by muscle, though the elastic lamina is continuous. There is a shallow bulge in the thin wall but no true aneurysm. That one had not developed may be due to the lateral position of the branch, away from the main force of the blood stream.

Other aneurysms from the sides of the cerebral arteries, not at recognizable forks, fall into a different category from those just described. These may be explained as derangements of some of the

innumerable minute arteries and precapillary vessels, only visible microscopically, which remain as persistent members of the original capillary plexus in the embryo from which the cerebral system has developed. These vessels are much more numerous than is usually recognized and are not adequately described in the textbooks; they serve as vasa vasorum for the larger vessels or supply the tissues of the local meninges. They arise, usually at right angles, directly from the main arteries, piercing the musculature as endothelial tubes surrounded by a minimal amount of connective tissue, or through larger gaps between the muscle bundles, in which case they assume their own slight musculature in the passage. They often retain their plexiform character just outside the vessel wall, branching in all directions. During early development many of the members of the net degenerate and are lost; usually one or two capillaries persist and enlarge, continuing the main stem. Occasionally all the capillary branches remain small or even undergo late degeneration. In the latter condition the combined outflow from the stem becomes less than the inflow from the parent artery. Such a case is shown in figure 9, from an embryo of 45 mm. The main stem, springing at right angles from the anterior cerebral artery, could receive more blood through its relatively wide entrance than could be transmitted through its three minute branches and has responded by dilating, since its walls are still of only capillary thickness. If this process had continued to adult life, an aneurysm would have resulted directly comparable to one described by Forbus (his no. 4) in the same position, with a narrow pedicle and two or three minute vessels branching from it at recurrent angles. The adventitia found in the wall of his specimen probably represents the usual reaction of the surrounding connective tissue to any expanding structure, a type of capsule formation; the muscularis and elastica had never developed on the original capillary.

Another similar condition is given in figure 4, the enlargement of a portion of figure 3, showing the reduction of the original plexus at the base of the posterior cerebral artery to a single stem. The stump of one of the original roots remains large though attached to only a single shriveled branch. It might well be considered as a presumptive aneurysm. Similar plexuses of tiny vessels, some members of which are still in the degenerative stage, are found not infrequently also at the forks of the larger arteries in the adult on microscopic examination. It is probable, therefore, that some of the aneurysms at the forks may be caused by the late irregular degeneration of these plexuses instead of by the pouching of Forbus' "medial defects." Their origin might be disclosed by the presence of minute vessels springing from some portion of their walls.

Analogous to this group are the aneurysms of the anterior communicating artery. This misnamed structure is seldom a single vessel.

Much more often it is composed of several separate parts, variously connected. De Vries [14] gave diagrams of many patterns found in man, all indicating its derivation from an original capillary plexus. In the embryo and the fetus the plexus is always present. In an infant of 1 week, though to the naked eye the connection appeared as a simple H, microscopic examination revealed the pattern of a three-dimensional net (fig. 10), in which certain members are favored, others reduced to microscopic dimensions and many others probably already lost. At *a* and at *b* conditions have arisen similar to those shown in figures 9 and 4, in which a segment of the plexus has been left with a wide entrance and a smaller exit and therefore has already become pouched and is liable to future aneurysmal enlargement, whether from increasing blood pressure or from an alteration in the direction of the stream as this flows by the open mouth, due to subsequent changes in the pattern or to the progressive coiling of the basal arteries often met in older persons. The latter might explain the late recognition of such aneurysms clinically.

A characteristic of true cerebral aneurysms is the absence of the internal elastic membrane as well as of the muscularis. The "medial defects" of Forbus and of Richardson and Hyland retain elastica in spite of the absence of media. Forbus explains its later disappearance as due to continued overstretching; the other authors see the necessity of a superadded lesion to weaken the elastica locally. Both agree that the resulting aneurysms are thus not strictly congenital, since the elastic tissue is lost after birth. This objection does not hold for some of the minute aneurysms shown in this paper.

Benninghoff,[15] in his review of vasculogenesis in man, showed that the elastic plate is first recognizable in the aorta of embryos of 3.5 cm. but that it is not recognizable in the radial artery until the length of the embryo is nearly 9 cm. He pointed out that the process proceeds centrifugally from the aorta. In the absence of any statement concerning the cerebral arteries one may safely place them nearer the latter in point of time, well beyond the age of the embryos in which were found the conditions shown in figures 4, 7 and 9. These, then, may be taken as presumptive aneurysms in which no elastica is present and therefore as true congenital aneurysms. As to whether these would later have assumed the elastic coat, one may be guided by the ideas of Roux,[16] who postulated that the type of vessel wall depends not on the

14. De Vriese, B.: Arch. di biol. **21**:357, 1905.

15. Benninghoff, A.: Blutgefässe und Herz, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 6, pt. 1.

16. Roux, W.: Theorie der Gestaltung der Blutgefässe, in Oppel, A.: Ueber die gestaltliche Anpassung der Blutgefässe, Leipzig, W. Engelmann, 1910.

size of the vessel nor on the internal pressure of the blood but on the relative strength of the pulse. This theory has been supported by Benninghoff and Spanner [17] in their description of an acardia, a twin fetus with complete absence of heart. The single umbilical artery was continuous across the surface of the placenta with one of the umbilical arteries of the normal twin. The aorta of the acardia thus became an end artery of the twin with reversed current and had all the characteristics of such an artery, with little elastic tissue. To follow this idea further, a blind pouch lying at the side of the blood stream might well be enlarged by the blood pressure and yet, since it transmits no pulsation, develop none of the usual components of an arterial wall. This same principle may even be applied to the walls of pouches which used to transmit pulsation and therefore acquired arterial coats (as in figure 10) but which lost their distal branches at an early age; under such circumstances the elastic tissue might disappear and allow further dilatation. This sequence would render unnecessary the introduction of a pathologic process or dependence on the doubtful theory that an elastic sheet can be worn out by continued overstretching. The aneurysms would be classed as spontaneous, though due to conditions similar to those leading to true congenital aneurysms.

SUMMARY

The cerebral arteries are evolved from a capillary plexus arising from the earliest branch of the primitive aortic arch, which runs along the under side of the brain. From the rostral end of the plexus branches go in front of the bulging hemisphere and over its lateral surface, becoming the anterior and middle cerebral arteries. Another part of the plexus becomes the posterior cerebral artery, covering the diencephalon and midbrain. The anterior artery gains the mesial surface of the frontal lobe; the posterior supplies the similar surface of the posterior lobes as their expansion covers in the diencephalon. All cerebral arteries approach from the lesser curvature of the expanding hemisphere, and the interstitial growth of the latter during fetal life rapidly spreads the forks of their branches. Other growth changes have the same action. If these forks lack the media, the rapid spread may produce local aneurysms.

From all the cerebral arteries and from the main basal trunk smaller branches dip into the brain substance and also supply the meninges. These also form plexuses. Proximal members of such plexuses may enlarge while their distal continuations degenerate and may thus become aneurysmal pouches from the main vessels. Both types may be true congenital aneurysms.

17. Benninghoff, A., and Spanner, R.: Morphol. Jahrb. **61**:380, 1929.

EPICELLULAR AND PERICELLULAR DEPOSITIONS OF AMYLOID AS THE STARTING POINT OF AMYLOIDOSIS

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Recently Pearson and associates [1] described pericellular amyloid rings in the adipose tissue of the adrenal glands in primary systemic amyloidosis. These amyloid rings were found only in the adipose tissue and not in the adrenal tissue proper. The authors considered such rings as "highly characteristic" of primary amyloidosis, of which only 28 cases have been described. I have observed such deposits of amyloid in the adrenal cortex in secondary, as well as in primary, amyloidosis. Amyloidosis is not a rare disease in Holland and I obtained material for study from the departments of pathology of Dutch universities and hospitals. To detect amyloid the methyl violet, iodine green and iodine–sulfuric acid reactions were used.

Of 12 instances in which amyloidosis of the adrenal gland was found, at least 9 were cases of advanced tuberculosis. In 3 cases the amyloidosis appeared to be primary. In 10 cases the epicellular and pericellular depositis in the adrenal cortex predominated.

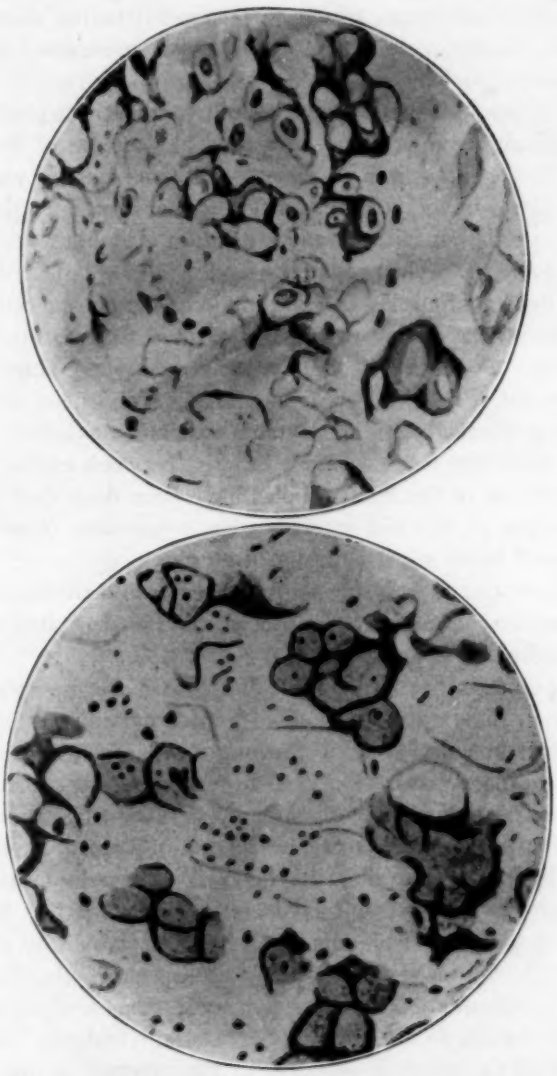
CASE 1.—The adrenal glands of a patient with pulmonary tuberculosis and systemic amyloidosis contained much amyloid chiefly in the fasciculate and reticular zones. The amyloid was deposited partly around cells and partly on capillaries. The figure illustrates the appearances in the reticular zone. The pericellular deposit of amyloid is shown clearly. A number of cells are surrounded by amyloid rings, which may be fused with the rings about adjacent cells.

CASE 2.—The adrenal glands of an 18 year old youth with tuberculosis of the lungs, bones and intestines contained large quantities of amyloid in the form of pericapillary and pericellular deposits. The amyloid stained a beautiful blue with iodine–sulfuric acid, especially after fixation in alcohol, suggesting that the amyloid was more "mature" than in other cases.

In only 2 cases was the pericapillary deposit of amyloid preponderant. In the other cases the cellular deposits predominated. Complete rings and shells of amyloid around cells could be observed. The shells did not always have the same thickness at all points. The inner surface of each shell was smooth and less deeply colored with iodine green than other areas. When only part of the cell was covered with amyloid, the deposit was designated as epicellular. The cells entirely enveloped in

1. Pearson, B.; Rice, M. M., and Dickens, K. La V.: Arch. Path. **32**:1, 1941.

amyloid appeared to undergo gradual atrophy, leaving either empty amyloid shells or solid spheres of amyloid. Some of the empty cavities might have been artefacts due to falling out of cells during the prep-



Upper circle: Pericellular deposits of amyloid. When the objective of the microscope was moved up and down, all rings appeared to be sections of shells. Stained with iodine green.

Lower circle: Amyloid deposits surrounding a number of cells. Note the melting together of the amyloid shells. Stained with iodine green.

aration of the microscopic sections. This possibility seems to be rather slight, however, since I used paraffin sections fixed on cover

glasses. Occasionally cells were found which were smaller than the cavities, and perhaps the atrophy was due more to lack of nourishment than to pressure. That amyloid may be deposited on the inside of the shell is suggested from the fact that the solid spheres of amyloid nearly all had the same diameter, which corresponded to the diameter of the cortical cells. When such spheres had become fused, their contours frequently remained visible.

In other tissues, also, I found amyloid frequently deposited on cells, and it is difficult to understand why the old doctrine of the deposition of amyloid in the interstices of the mesenchymal tissues was not abandoned long ago. In the literature references are made to the deposition of amyloid on cells but without any indication of doubt as to the validity of the old doctrine. Thus, Benecke and Bönning [2] described amyloid deposits in the heart muscles as a walling in of the muscle cells. Hueter [3] wrote that in cartilage the amyloid is deposited first on the walls of the cartilage cells. Spronck [4] in 1919 observed amyloid rings around fat cells. As stated, Pearson, Rice and Dickens [1] described amyloid rings in fat tissue as characteristic of primary systemic amyloidosis. Amyloid deposits on epithelial cells or, more exactly, between epithelial cells and the tunica propria of the renal tubules have been described. The epicellular localization of amyloid in other glands has been observed. In the intima of small veins small deposits of amyloid may occur on epithelial cells, sometimes causing these cells to protrude into the lumen. In connective tissue and other tissues amyloid may be deposited on capillary endothelial cells.

Several points in the localization of amyloid are unexplained by the old doctrine. The pericapillary deposit of amyloid was looked on as the result of transudation of a precursor of amyloid from the blood through the capillary walls. The cardiac valves and the chordae tendineae have no blood vessels, however, and, in spite of that, ramifying amyloid deposits may occur. Here the theory of transudation fails completely. Schmidt [5] noted that the deposits of amyloid in the cardiac valves are covered with endothelial cells. May not this be a deposit of amyloid on the cells of lymph vessels? Unexplained by the theory of transudation is the fact that in arteries, especially the larger, where a stream of transudation can hardly occur, deposits of amyloid are found in the media in the early stages of amyloidosis. Unexplained also are branching amyloid deposits in the arterial media. Schmidt [5] found that in the media of small arteries deposits of amyloid occupy

2. Benecke, R., and Bönning, L.: Beitr. z. path. Anat. u. allg. Path. **44**: 362, 1908.

3. Hueter, C.: Beitr. z. path. Anat. u. z. allg. Path. **49**:100, 1910.

4. Spronck: Personal communication to the author.

5. Schmidt, M. B.: Verhandl. d. deutsch. path. Gesellsch. **7**:2, 1904.

the position of muscle cells. Here again deposition of amyloid on the muscle cells would explain the situation. A disputed point has been the difference in location of amyloid deposits in connective tissue. In fibrous connective tissue the amyloid is not deposited on the fibers as it is in reticular connective tissue. The explanation seems to be simple: In fibrous connective tissue the fibers are not covered with cells, but in reticular connective tissue the fibers and also the processes are covered with cells, as described by Pekelharing,[6] on which amyloid is deposited.

SUMMARY

The deposition of amyloid on cells rather than deposition in the interstices of mesenchymal tissue may be the starting point of amyloidosis.

6. Pekelharing, C. A.: Voordrachten over weefselleer, Haarlem, F. Bohn, 1905, p. 225.

EPINEPHRINE AND RELATED SUBSTANCES IN HUMAN ARTERIAL WALLS AND KIDNEYS

THEIR ROLE IN ARTERIOSCLEROSIS

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Arteriosclerosis is often referred to as a vascular condition produced by a "damaging agent" of unknown nature.[1] Little attention has been paid so far to the fact that the body contains a substance which is known to cause severe vascular damage if administered or secreted in abnormally large amounts, namely, epinephrine, the most thoroughly studied among the hormonal substances which are manufactured and secreted by the medulla of the adrenal gland. A very similar, if not identical, substance, sympathin, is liberated by peripheral sympathetic neurons in the vascular walls themselves.[2]

That epinephrine and related substances play an outstanding role in the development of arteriosclerosis and arteriolosclerosis is suggested by the following facts:

(*a*) In animals repeated injections of epinephrine hydrochloride produce severe medial changes in arteries, analogous to those found in human arteriosclerosis.[3]

(*b*) In rabbits experimental cholesterol lipoidosis of the intima was found to be greatly enhanced by injections of epinephrine [4] and of lipoid extracts of the adrenal glands.[5a]

(*c*) Epinephrine is absorbed by arterial tissue in vitro.[6]

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1. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935. Jobling, J. W., and Meeker, D. R.: ibid. **22**:293, 1936.

2. (*a*) Gaddum, J. H., and Kwiatkowski, H.: J. Physiol. **96**:385, 1939. (*b*) Cannon, W. B., and Lissák, K.: Am. J. Physiol. **125**:765, 1939.

3. Josué, O.: Compt. rend. Soc. de biol. **55**:1374, 1903. Hesse, M.: Virchows Arch. f. path. Anat. **249**:437, 1924.

4. Danisch, F.: Beitr. z. path. Anat. u. z. allg. Path. **79**:333, 1928. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

5. Raab, W.: (*a*) Ann. Int. Med. **14**:1981, 1941; (*b*) Arch. Int. Med. **68**: 713, 1941; (*c*) J. Clin. Endocrinol. **1**:977, 1941; (*d*) Endocrinology **28**:325, 1941; (*e*) **29**:126 and (*f*) 564, 1941; (*g*) Am. Heart J. **24**:365, 1942; (*h*) Exper. Med. & Surg. **1**: no. 2, 1943; (*i*) Endocrinology **32**:226, 1943.

6. Tatum, A. L.: J. Pharmacol. & Exper. Therap. **4**:151, 1913.

(*d*) Repeated implantations of adrenal tissue cause vascular changes similar to arteriolosclerosis.[7]

(*e*) Arteriosclerosis and arteriolosclerosis, including nephrosclerosis, are common in persons with tumors of the adrenal glands,[8] even in infancy and youth.

(*f*) Most patients with pituitary disorders which are accompanied by hyperplasia of the adrenal glands show arteriosclerotic changes.[9]

(*g*) Clinical conditions which are intimately connected with arteriosclerotic changes, such as essential hypertension and angina pectoris, have been found to be characterized by abnormally intense temporary discharges of epinephrine and epinephrine-like substances into the blood stream.[5b, c]

(*h*) The heart muscles of most persons who died from "hypertensive" and "arteriosclerotic" heart disease were found to contain abnormally high concentrations of epinephrine and of epinephrine-like substances.[5h]

In view of the facts just mentioned it appeared desirable to determine the quantitative concentration of epinephrine and related substances in human arterial walls and kidneys.

METHOD AND MATERIAL

Shaw's colorimetric method for the determination of epinephrine[10] as modified by me[5d, e] was used. Other substances, besides epinephrine proper, which participate in the results obtained with this method are related compounds with a catechol nucleus (such as adrenalone, dihydroxyphenylalanine, leukoadrenochrome, sympathin) and ascorbic acid.[5i] The adrenal medulla itself was found to contain relatively large amounts of such substances besides epinephrine proper.[5i]

The term "AC" (from "adrenal catechols") will be used in the tables and chart to designate the total content of chromogenic material described in the foregoing paragraph (i. e., epinephrine, other adrenal catechols and ascorbic acid) on which the colorimetric result in each case was obtained. (In several previous publica-

7. Leriche, R., and Froelich, F.: Ann. d'anat. path. **13**:1039, 1936. Hornowski, J.: Virchows Arch. f. path. Anat. **215**:280, 1914. Maggi, N., and Mazzocchi, E.: Arch. ital. di chir. **35**:369, 1933.

8. (*a*) Paul, F.: Virchows Arch. f. path. Anat. **282**:256, 1931. (*b*) Biebl, M., and Wichels, P.: ibid. **257**:182, 1926; München. med. Wchnschr. **75**:656, 1928. (*c*) Kremer, D. N.: Arch. Int. Med. **57**:999, 1936. (*d*) Hegglin, R., and Nabholz, H.: Ztschr. f. klin. Med. **134**:161, 1938. (*e*) Mainzer, F.: Acta med. Scandinav. **87**:50, 1935. (*f*) Moltschanoff, W. J., and Davydowski, J. W.: Virchows Arch. f. path. Anat. **274**:606, 1930. (*g*) Büchner, F.: Klin. Wchnschr. **17**:617, 1934. (*h*) Wells, H. G., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933. (*i*) Fuller, R. H.: Arch. Path. **32**:556, 1941.

9. McMahon, H. E.; Close, H. G., and Hass, G.: Am. J. Path. **10**:177, 1934. Kessel, F. K.: Ergebn. d. inn. Med. u. Kinderh. **50**:620, 1936. Raab.[5a]

10. Shaw, F. H.: Biochem. J. **32**:19, 1938.

tions [11] that abbreviation was used for "adrenocortical" compounds in the erroneous belief that cortical steroids participate directly in the colorimetric results, owing to compound formation with epinephrine. This conception was later abandoned, however, when it was found that such steroids, although altering the chromogenic and biologic properties of epinephrine,[5f,g] do not unite with it in water-soluble compounds.)

The colorimetric readings are expressed in color units per gram of fresh tissue, each of which corresponds to the color intensity of 10^{-6} mg. of pure epinephrine. A rough evaluation of the qualitative composition of the total chromogenic material is possible through determination of the "specific ratio," [12] the denominator of which (d.s.r.) varies with the relative amount of epinephrine and of the other constituents of the total material. A denominator from 2.00 upward indicates prevalence or exclusive presence of epinephrine (and sympathin); lower figures between 2.0 and 1.0 indicate increasing participation of other, epinephrine-like substances, and an occasional denominator lower than 1.0 is attributable to prevalence of ascorbic acid.

The ascending parts of 42 aortas, 12 renal arteries and 42 kidneys (cortical tissue) were examined within a few hours after autopsy, during which time the tissues were kept in saline solution in the refrigerator. Immediately before chemical examination the arteries were stripped of their adventitia and the kidneys of their capsule. From 400 to 800 mg. of tissue was superficially dried with filter paper, weighed and worked up as described in previous publications.[5d, e] The fact that the material was not available immediately after death possibly accounts for some of the lower figures for total chromogenic material, since a slow loss of color takes place in the dead tissues. The denominator of the specific ratio remains practically unchanged, however.

RESULTS

Aorta.—In table 1 37 aortas of adult persons are grouped according to absence or presence of macroscopically detectable arteriosclerosis. In these two groups, as well as in a few infantile aortas, the majority of the denominators were far above 2.00, indicating the presence of pure epinephrine or of a substance closely related to it, probably sympathin.

The average readings of total chromogenic material (AC in table 2) were about the same in the nonsclerotic and in the sclerotic aortas of adults. However, the presence of chromogenic material other than epinephrine proper and sympathin (indicated by a denominator lower than 2.00) was more frequently encountered in arteriosclerotic aortas, particularly in those with sclerosis of higher degrees. Excessively high concentrations of chromogenic material (above 1,000 color units per gram) were present in 3 aortas of the latter group (cases 26, 31 and 37). The highest value was found in a case in which an adenoma of the adrenal cortex extended into the medulla (case 31).

When all aortas were grouped according to age (table 2), a marked increase of the values for total chromogenic material was noted with age,

11. Raab (footnotes 5 *b*, *c*, *d*, *e* and *f*).

12. Shaw.[10] Raab.[5f]

TABLE 1.—*Cases in Which Aorta Was Studied*

Case	Sex	Age	Degree of Arterio-sclerosis	Blood Pressure	AC, Color Units per Gm.*	Denominator of Specific Ratio †	Diagnosis
				Normal Aorta			
1	♂	19	..	126/ 84	83	>4.00	Chronic heart failure
2	♀	20	..	120/ 50	715	>4.00	Chronic heart failure
3	♀	27	..	112/ 74	622	2.20	Carcinoma of uterus
4	♀	38	..	165/ 90	656	>4.00	Uremia
5	♀	39	..	110/ 80	Trace	>4.00	Pneumonia
6	♂	42	..	112/ 84	567	4.00	Peritonitis
7	♀	43	..	210/140	426	>4.00	Chronic heart failure
8	♀	50	..	230/100	610	>4.00	Cerebral hemorrhage
9	♀	51	..	.....	585	1.27	Intestinal hemorrhage
10	♀	51	..	120/?	Trace	>4.00	Appendicitis
11	♀	58	..	130/100	773	1.52	Carcinoma of the breast
12	♂	63	..	90/ 50	625	1.41	Carcinoma of the sigmoid
13	♀	69	..	110/ 65	585	>4.00	Pernicious anemia; pneumonia
14	♀	71	..	112/ 72	518	>4.00	Sarcoma of small intestine
15	♂	78	..	160/ 70	739	>4.00	Chronic heart failure
16	♀	81	..	160/ 72	775	2.45	Chronic heart failure
17	♀	92	..	160/ 72	104	>4.00	Senility
				Sclerotic Aorta			
18	♂	37	+	.....	163	>4.00	Cerebral hemorrhage
19	♀	46	+	100/ 80	195	3.80	Cerebral hemorrhage
20	♂	55	+	90/ 52	100	>4.00	Chronic heart failure
21	♂	62	+	.....	686	3.28	Carcinoma of the sigmoid
22	♂	63	+	246/100	151	3.80	Surgical shock
23	♂	69	+	100/ 56	532	>4.00	Pulmonary embolism
24	♂	74	+	142/ 76	548	1.91	Carcinoma of cecum
25	♂	83	+	150/ 62	Trace	>4.00	Pneumonia
26	♂	39	++	238/144	1,278	1.35	Uremia
27	♀	48	++	182/116	58	>4.00	Cerebral hemorrhage
28	♂	58	++	110/ 50	752	1.75	Intestinal hemorrhage
29	♂	58	++	225/130	470	1.56	Uremia; nephrosclerosis
30	♂	63	++	100/ 66	375	2.30	Carcinoma of bladder
31	♂	68	++	138/ 20	1,993	1.43	Adrenal cortical adenoma
32	♂	68	++	160/ 60	15	3.00	Subarachnoidal hemorrhage
33	♂	69	++	140/ 90	Trace	>4.00	Congestive heart failure
34	♀	72	++	218/ 74	751	>4.06	Carcinoma of stomach; adenoma of adrenal medulla
35	♂	76	++	.....	355	1.49	Chronic heart failure
36	♀	81	++	140/ 70	202	3.80	Senility
37	♂	50	+++	"Low"	1,062	1.58	Coronary sclerosis
				Infantile Aorta			
38	♀	48 hr.	.....	.....	Trace	>4.00	Hepatic hemorrhage
39	♂	2 mo.	.....	.....	561	.....	Otitis media
40	♀	1 yr.	.....	.....	Trace	>4.00	Marasmus
41	♂	5 yr.	.....	.....	220	3.53	Meningitis
42	♂	7 yr.	.....	.....	82	3.60	Cerebral tumor

* These figures represent the total colorimetric readings (adrenal catechols, sympathin and ascorbic acid) for which the term AC is used.

† The denominator of the specific ratio (d. s. r.) characterizes the qualitative composition of the total AC material. A d. s. r. from 2.00 upward indicates the prevailing or exclusive presence of epinephrine proper or of sympathin. A lower d. s. r. indicates the presence of other AC compounds.

TABLE 2.—*Average Values for Various Groups of Aortas*

	Cases	Average Age, Yr.	AC, Color Units per Gm.*	Percentage of Cases in Which D. S. R.† Smaller Than 2.00 Was Found
Degree of arteriosclerosis				
None	17	52	493	18
Slight to severe	20	62	484	35
Age groups				
0 to 7 years	5	2½	173	0
19 to 50 years	12	37	507	8
51 to 69 years	16	61	575	44
71 to 92 years	9	79	444	22
Systolic blood pressure				
90 to 150 mm. Hg	19	55	379	27
151 to 246 mm. Hg	13	62	616	18

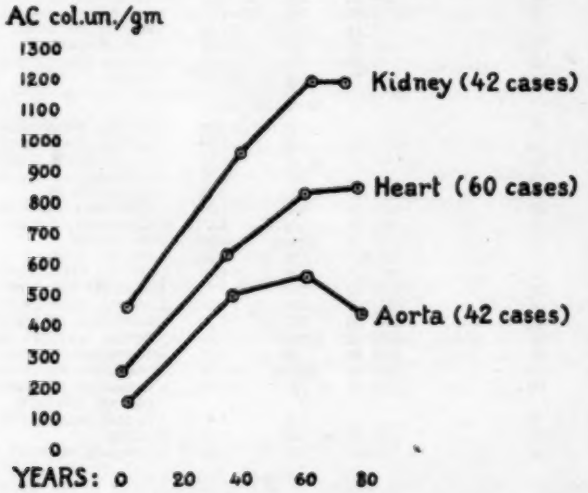
* See explanation under table 1.

† See explanation under table 1.

the peak occurring between 51 and 69 years, followed by a slight decline (figure). This age curve was roughly paralleled by the frequency of the presence of chromogenic substances other than epinephrine or sympathin.

The aortas of hypertensive persons (table 2) contained on an average larger amounts of chromogenic substances than those of nonhypertensive persons.

Renal Artery.—No significant difference of the average concentration of the total chromogenic material was found between 8 nonsclerotic and 4 sclerotic renal arteries (table 3). The values were generally lower than



The increase of the concentration of the total chromogenic material (*AC*-epinephrine, other adrenal catechols and ascorbic acid) with age in the human aorta, heart and kidney (expressed in color units per gram).

TABLE 3.—*Cases in Which Renal Artery Was Studied*

Case	Sex	Age	Degree of Arterio-sclerosis	Blood Pressure	AC, Color Units per Gm.*	Denominator of Specific Ratio †	Diagnosis
				Morphologically Normal Renal Arteries			
43	♂	49	..	98/ 70	121	3.80	Carcinoma of cystic duct
44	♂	50	..	130/ 80	39	>4.00	Pulmonary embolism
8	♀	50	..	230/100	61	>4.00	Cerebral hemorrhage
28	♀	58	..	130/100	86	>4.00	Carcinoma of the breast
11	♂	58	..	110/ 50	296	2.00	Intestinal hemorrhage
16	♂	60	..	160/ 72	675	2.46	Chronic heart failure
21	♂	62	..	.....	Trace	>4.00	Carcinoma of sigmoid
31	♂	68	..	138/ 20	784	>4.00	Adrenal cortical adenoma
Average		57	..	142/ 70	258		
				Sclerotic Renal Arteries			
24	♂	74	++	142/ 76	387	>4.00	Carcinoma of cecum
35	♂	76	++	.....	505	3.60	Chronic heart failure
20	♂	55	++	90/ 50	Trace	....	Chronic heart failure
25	♂	83	++	150/ 62	47	0.71	Pneumonia
Average		72	....	127/ 63	257		

* See explanation under table 1.
† See explanation under table 1.

those for the aorta. The highest reading was obtained in a case of adenoma of the adrenal cortex (case 31). With one exception all renal arteries contained pure epinephrine or sympathin.

TABLE 4.—*Cases in Which Kidneys Were Studied*

Case	Sex	Age	Degree of Nephrosclerosis	Blood Pressure	AC, Color Units per Gm.*	Denominator of Specific Ratio †	Diagnosis
				Morphologically Normal Kidneys			
2	♀	20	..	120/ 50	1,368	1.22	Chronic heart failure
6	♂	42	..	112/ 84	835	1.01	Peritonitis
19	♀	46	..	100/ 80	1,684	1.48	Cerebral hemorrhage
43	♂	49	..	98/ 70	767	1.43	Carcinoma of cystic duct
44	♂	50	..	130/ 80	1,065	0.91	Pulmonary embolism
11	♀	58	..	130/100	900	1.19	Carcinoma of breast
21	♂	62	..	.....	641	1.44	Carcinoma of sigmoid
32	♂	68	..	100/ 60	481	0.69	Subarachnoidal hemorrhage
31	♂	68	..	138/ 20	1,795	1.86	Adrenal cortical adenoma
34	♀	72	..	218/ 74	2,947	1.00	Carcinoma of stomach; adenoma of adrenal medulla
1	♂	19	..	126/ 84	1,110	2.27	Chronic heart failure
45	♂	63	..	120/ 80	4,469	1.11	Heart failure; albuminuria ++
33	♂	69	..	140/ 90	579	1.33	Chronic heart failure
24	♂	74	..	142/ 76	1,495	1.60	Carcinoma of cecum; albuminuria +
				Kidneys with Arteriolosclerosis			
10	♀	51	+	120/ ?	971	1.19	Peritonitis
20	♂	55	+	90/ 62	220	3.70	Chronic heart failure
13	♀	69	+	110/ 65	1,051	1.27	Pneumonia; albuminuria +
16	♀	81	+	160/ 72	2,223	1.06	Chronic heart failure
17	♀	92	+	160/ 72	Trace	....	Senility
18	♂	37	++	.....	461	4.20	Cerebral hemorrhage
7	♂	43	++	210/140	1,621	1.87	Chronic heart failure
27	♀	48	++	182/116	459	1.73	Cerebral hemorrhage
29	♂	58	++	225/130	1,708	1.17	Uremia; albuminuria +
22	♂	63	++	246/100	595	1.88	Surgical shock
23	♂	69	++	100/ 56	1,083	3.11	Pulmonary embolism
35	♂	76	++	.....	439	0.91	Chronic heart failure; albuminuria +
36	♀	81	++	140/ 70	812	1.39	Senility
46	♂	82	++	160/ 92	1,176	1.52	Concussion of brain
25	♂	83	++	150/ 62	549	0.65	Pneumonia
8	♀	50	+++	230/100	427	4.20	Cerebral hemorrhage; albuminuria ++
47	♀	67	+++	100/ 50	293	3.60	Chronic heart failure; albuminuria ++
15	♂	68	+++	160/ 70	739	4.30	Chronic heart failure; albuminuria ++
				Infantile Kidneys			
48	♂	9 hr.	.....	.....	76	4.10	Atelectasis
49	♂	13 hr.	.....	.....	315	4.04	Hemorrhage left adrenal gland
50	♂	40 hr.	.....	.....	139	2.02	Atelectasis
38	♀	48 hr.	.....	.....	1,530	0.70	Hepatic hemorrhage
51	♀	7 wk.	.....	.....	408	....	Colitis
39	♂	2 mo.	.....	.....	681	0.95	Otitis media
40	♀	1 yr.	.....	.....	326	1.32	Marasmus
52	♂	1½ yr.	.....	.....	42	3.91	Meningitis
41	♂	5 yr.	.....	.....	386	3.06	Meningitis
42	♂	7 yr.	.....	.....	882	1.47	Tumor of brain

* See explanation under table 1.
† See explanation under table 1.

Kidney.—Forty-two kidneys (including 10 infantile ones) were grouped in the same manner as the aortas (table 4). The qualitative composition of the chromogenic material in the kidney was generally quite different from that in the arterial walls in that the material other than epinephrine proper or sympathin was prevalent in most specimens

deriving from adults. The presence of pure epinephrine or sympathin, although common in infantile kidneys, was confined in the groups of adults almost entirely to those kidneys which showed marked arteriolosclerotic changes. The average values for the total chromogenic material increased with age (table 5; figure), and also chromogenic material other than epinephrine was more commonly found in adults than in children, reaching a maximum occurrence in senile persons (100 per cent).

In those cases in which there had been marked albuminuria during life, there was either an abnormally high concentration of total chromogenic material (case 45) or an abnormally high concentration of epinephrine proper in the kidneys (cases 8, 47 and 15). The second highest concentration of the total chromogenic material was found in a case of

TABLE 5.—*Average Values for Various Groups of Kidneys*

	Cases	Average Age, Yr.	AC, Color Units per Gm.*	Percentage of Cases in Which D. S. R.† Smaller Than 2.00 Was Found
Degree of arteriosclerosis				
None	14	54	1,439	93
Slight to severe	18	65	810	65
Age groups				
0 to 7 years	10	1½	479	44
19 to 50 years	10	40	980	70
51 to 69 years	14	63	1,208	71
71 to 92 years	8	74	1,205	100
Systolic blood pressure				
90 to 149 mm. Hg	18	57	1,166	78
150 to 246 mm. Hg	11	67	1,131	82

* See explanation under table 1.
† See explanation under table 1.

tumors of the adrenal medulla (case 34). High amounts of the total chromogenic material were also present in the kidney in a case of uremia (case 29) and in a case of adenoma of the adrenal cortex (case 31).

COMMENT

Among the epinephrine-like substances which, together with epinephrine proper, sympathin and ascorbic acid, participate in the color reaction used in the determinations discussed in the foregoing section there are catechol compounds which exert certain pharmacodynamic effects on the cardiovascular system [13] and which are eagerly absorbed by the heart muscle.[5f,1] Their possible effects on arterial and renal morphologic structure have not yet been studied. However, the abnormally high concentrations of epinephrine-like catechols not identical with epinephrine which were almost regularly found in the blood [5b] and the

13. (*a*) Tainter, M. L.: J. Pharmacol. & Exper. Therap. **40**:43, 1930. (*b*) Tani, S.: Folia pharmacol. japon. **13**:393, 1932. (*c*) Oster, K. A.: Nature, London **150**:289, 1942.

heart muscle [5h] of uremic patients are paralleled by particularly severe myocardial lesions in uremia.[14] This seems to indicate that other, epinephrine-like substances may be at least as detrimental to cardiovascular structural integrity as epinephrine itself.

The conversion of epinephrine and of its precursors into other pharmacodynamically vasoactive compounds takes place through the action of certain enzymes (amino oxidases, catecholases and so on [15]). Hormonal steroids, particularly those having their origin in the adrenal cortex, modify both the chromogenic and the biologic properties of epinephrine.[16]

With these facts in mind, it will be realized that the amounts and distribution of epinephrine, sympathin and other epinephrine-like substances in arterial walls, kidneys and other tissues will depend not solely on the intensity of adrenal secretion but also on a variety of local neurohormonal and enzymatic processes, presence of certain lipids and other factors. The degree of the destructive effect on vascular tissues will probably likewise be influenced by these factors.

Nevertheless, it seems significant that particularly high concentrations of the chromogenic material were found in the arteries and kidneys of persons with adrenal tumors.

The presence of pure epinephrine or sympathin is much more common in the tissue of the aorta and the renal artery than in the kidney. This may be due to the fact that epinephrine is excreted through the kidneys in a modified form [17] and perhaps also to the presence of lipids in the kidney [8l] which are likely to modify the epinephrine molecule.

Age is a determining factor in the vascular and renal distribution of the total chromogenic material: The lowest average values for the total material were found for the aortas and kidneys of infants and young children and the highest ones for those in the sixth and the seventh decade of life. Also the accumulation of the chromogenic substances other than epinephrine proper or sympathin increases distinctly with age both in arterial walls and in kidneys. Analogous conditions, namely, low total chromogenic material and low nonepinephrine material in infancy and increase of both with age, have been observed in the human heart muscle.[5h] The changes in the total chromogenic material of the

14. Gouley, B. A.: Am. J. M. Sc. **200**:39, 1940.

15. Bernheim, F.: The Interaction of Drugs and Cell Catalysts, Minneapolis, Minn., Burgess Publishing Company, 1942. Bing, R. J.: Am. J. Physiol. **132**: 497, 1941. Tani.[13b] Oster.[13c]

16. Sanders, E.: Arch. f. exper. Path. u. Pharmakol. **188**:657, 1938. Raab.[5l, g]

17. Richter, D.: J. Physiol. **98**:361, 1940.

aorta with age may possibly be related to the increasing deposition of lipids in the aortic walls with advancing age.[18]

In arteriosclerotic aortas abnormally high values both for the total chromogenic material and for the accumulation of nonepinephrine material were encountered more frequently than in nonsclerotic aortas. Here again the modifying effect of steroids on properties of the epinephrine molecule may be of importance, as well as degenerative changes in the sympathetic ganglions which furnish the nerve supply to the arteriosclerotic vascular areas.[19] Experimental degeneration of sympathetic fibers was found to alter the chromogenic properties of epinephrine or of sympathin in the heart.[20]

Conditions in the arteriosclerotic kidneys were different from those in the aortas so far as the average total chromogenic material was somewhat lower in the sclerotic kidneys than in the morphologically normal ones. Only the infantile and the sclerotic kidneys contained pure epinephrine in a significant number of instances. Analogous conditions were observed by me in the human heart; pure epinephrine was found almost exclusively in infantile hearts and in those of persons who had died from cardiac failure with myocardial hypertrophy and degeneration.[5b] Thus, the behavior of the prevailingly muscular renal arterioles in regard to storage and formation of the chromogenic material seems to resemble more that of the heart muscle than that of the elastic fibrous aorta.

Marked albuminuria seems to be connected with high renal concentrations of either the total chromogenic material or epinephrine proper. Experimental albuminuria has been produced by the administration of epinephrine.[21]

In view of the fact that epinephrine elevates the renal threshold for excretion of sugar,[22] the high concentrations of epinephrine observed in infantile and sclerotic kidneys may be regarded as responsible for the high

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18. Meeker, D. R., and Jobling, J. W.: Arch. Path. **18**:252, 1934. Weinhouse, S., and Hirsch, E. F.: ibid. **29**:31, 1940. Bruger, M., and Chassin, M. R.: Ann. Int. Med. **14**:1756, 1941. Bürger, M.: First International Congress of the Union of Therapeutics, Berne, H. Huber, 1938.

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20. Bacq, Z. M.: Arch. internat. de physiol. **36**:167, 1933. Cannon.[2b]

21. Bornstein, A.: Klin. Wchnschr. **1**:1484, 1922. Tomaszewski, Z.: Deutsches Arch. f. klin. Med. **124**:394, 1918. Zuelzer, F.: Berl. klin. Wchnschr. **38**:1209, 1901. Goldzieher, M., and Molnar, B.: Wien. klin. Wchnschr. **21**:215, 1908.

22. Dillon, T. W. T., and Feric, S.: Proc. Roy. Irish Acad. **47**:179, 1941.

dextrose threshold which has been found to be characteristic of infants [23] as well as of diabetic patients with nephrosclerosis.[24]

No clear relations were observed in regard to the concentration of total chromogenic material in the arteries and kidneys of nonhypertensive and hypertensive persons except for a somewhat higher content of the chromogenic material in the aortas in the latter group.

SUMMARY AND CONCLUSIONS

Epinephrine and epinephrine-like substances (adrenal catechols) were determined colorimetrically in human aortas, renal arteries and kidneys.

Infantile tissues contained the lowest total amounts of chromogenic material. It consisted almost entirely of epinephrine proper or sympathin.

With advancing age, increasing amounts of other, epinephrine-like substances, similar to those produced by the adrenal medulla, were found to accumulate in vascular walls and kidneys.

Abnormally large concentrations were observed in the vessels and the kidneys of persons with adrenal tumors.

Sclerotic aortas contained high concentrations of chromogenic material other than epinephrine proper more frequently than normal aortas.

In arteriosclerotic kidneys, on the other hand, pure epinephrine or sympathin was more commonly encountered than in morphologically normal kidneys. In this respect arteriosclerotic kidneys resemble the failing hypertrophic and damaged human heart.

In cases of marked albuminuria the renal concentrations of the total chromogenic material or of epinephrine proper were high.

The role of adrenal hormones and sympathin as intrinsic damaging agents in the origin of arteriosclerosis is discussed.

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EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

VII. CHRONIC NICOTINE POISONING IN RATS AND IN DOGS

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In the study of chronic nicotinism, especially as to its arteriosclerotogenic effects, uncertainty still prevails concerning the causative mechanism of the various vascular lesions observed. The controversy revolves about the question whether nicotine exerts a direct vasopressor action or an indirect one through influencing the vasomotor centers of the medulla oblongata or through causing a release of epinephrine from the adrenal glands (Straub and Amann [1]; Cannon, Aub and Binger [2]; Dale and Laidlaw [3]; Eichholtz [4]; Kosdoba [5]; Thienes, Lombard and Lesser [6]; Stätlander [7]), or elicits an allergy with the vascular system as the shock organ. The solution of this problem has been complicated by the fact that experimental studies on animals showed that degenerative arterial lesions could be elicited readily in rabbits by repeated subcutaneous, oral or respiratory introduction of nicotine or nicotine-containing agents (Josué [8]; Gouget [9]; Boveri [10]; Adler and Hensel [11]; Adler [12]; Baylac [13]; Lesieur [14]; Guillan and Gy [15]; von Zebrowski [16];

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(Footnotes continued on next page)

Gotsev [17]; Lee [18]; Schmiedl [19]; Kosdoba [5]; Krylow [20]; Sstarokadomsky [21]; Miller [22]; Papadia,[22] and others), while the arteries of rats similarly treated remained intact (Staemmler [22a]; Thienes and Butt [23]; Wilson, McNaught and DeEds [24]) in spite of the appearance of adenomatous proliferations in the medulla of the adrenal gland (Staemmler). This observation led Staemmler to the conclusion that rats are refractory to epinephrine and nicotine in vascular respects. The situation was further confused by the claim of McCormick [25] that subcutaneous administration of epinephrine prior to administration of nicotine to rabbits protects against fatal doses of this alkaloid. This contention was disputed in turn by Haag and Fisher [26] and Kin,[27] who reported that epinephrine accentuates the toxicity of nicotine.

The present investigations were undertaken in an attempt to clarify several aspects of the causative and protective mechanisms involved in the production of chronic nicotinism and its vascular effects.

EXPERIMENTS

Six mongrel dogs, approximately 3 months old and weighing from 2.3 to 3.6 kilograms, and 150 male and 30 female rats, 3 months old and weighing from 150 to 175 Gm., were used in these experiments.

The dogs as well as 30 male and 30 female rats received subcutaneous injections of solutions of chemically pure nicotine alkaloid (Eimer and Amend) only, at the rate of five injections a week. The dogs were given 0.7 cc. of an aqueous 1:1,000 solution of the nicotine alkaloid for one week, 1 cc. for four weeks, 2 cc. for three weeks, 3 cc. for three weeks, 5 cc. for four weeks, 5 cc. of a 1:660 solution for four weeks and 5 cc. of a 1:225 solution for two weeks. From then on, a 3 per cent solution of nicotine in peanut oil was used, and 1 cc. of this agent was given for three weeks and 2.5 cc. for five weeks. Four of the 6 dogs died during the first

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month of the experiment from intercurrent infections. The 2 surviving dogs were killed for histologic study at the end of an experimental period of ten months. The two series of rats which received nicotine alkaloid only were given 0.2 cc. of an aqueous 1 : 1,000 solution of this substance for a period of four weeks, 0.5 cc. for four weeks, 1 cc. of a dilution of 1 : 660 for four weeks, 1 cc. of a dilution of 1 : 225 for eleven weeks and 1 cc. of a dilution of 1 : 100 for five weeks. The surviving animals were killed for histologic study at the end of an experimental period of approximately eight months.

The remaining 120 male rats were divided into four series of 30 animals each. In addition to the treatment with nicotine as outlined in the foregoing paragraph, the following medication was given: One series of rats received, with their daily ration of 10 to 15 Gm. of McCollum's stock diet, 0.2 Gm. of ascorbic acid and 0.2 Gm. of cystine. A second series received, immediately after each injection of the nicotine solution, a subcutaneous injection of 0.1 cc. of a suspension of epinephrine in peanut oil (1 : 10,000). A third series had as supplementary treatment a subcutaneous injection of a solution of desoxycorticosterone acetate in peanut oil (1 : 2,500). (The desoxycorticosterone acetate was donated by Mr. E. H. Bobst, Roche-Organon, Inc.) The fourth series received a suspension of acetylbetamethylcholine chloride (mecholyl chloride) in peanut oil (1 : 5,000), 0.25 cc. of this material being injected subcutaneously directly after each introduction of the nicotine solution. After four weeks the concentration of the suspension of mecholyl chloride was increased to 1 : 400 and was kept at this level to the end of the experiment. All experiments were terminated after eight months, when the surviving rats were killed.

RESULTS

Symptoms.—Spastic convulsions developed in all rats within two minutes after each injection of the solution of nicotine. The convulsive state, which lasted about two to five minutes, was followed by a period of exhaustion and stupor, during which the hindlegs were paralyzed and the respiration was considerably increased. In rats which received an injection of a suspension of epinephrine in oil immediately after the administration of nicotine the onset of the convulsions was hastened and their degree was accentuated. However, preceding this reaction there was a stage of extreme excitation, during which the rats attempted to jump out of the container. The final reactive phase was again one of deep depression. The animals of this series did not gain in weight to any marked degree but remained rather small and skinny. They had rough fur and a haggard look. Their feet were cold and cyanotic to a higher degree than those of any of the other series.

The rats of the nicotine-mecholyl chloride series exhibited a similar primary excitation, during which their eyes became dark red and protruding and shed blood-tinged tears (chromodacryorrhexia). This phase lasted about ten to fifteen minutes and was followed by convulsions and finally by depression. The rats subjected to injections of solutions of nicotine and desoxycorticosterone acetate reacted like animals given nicotine only. In the rats which consumed a diet with additions of cystine and ascorbic acid, the onset of the convulsive stage was delayed, so that it occurred usually not before ten to fifteen minutes after the injection of the solution of nicotine, and the attack was less severe in character and shorter in duration than in animals kept on a stock diet.

The dogs usually vomited after the injection of a solution of nicotine and were either unable to walk or had lost proper control over their legs.

Mortality Rates.—These differences in symptomatic reactivity among the various series are reflected in part in their respective mortality rates, which are

presented in figure 1. The curves show clearly that the mortality rate for the animals which received the combined nicotine-epinephrine treatment was the highest, being closely followed by that of the series given the nicotine-desoxycorticosterone acetate and that of the series given the nicotine-mecholyl chloride treatment. The mortality curve of the female rats treated with nicotine only showed, following the eighteenth week of treatment, an abrupt break after having exhibited up to that time relatively low mortality. The increase in mortality among the female rats after the eighteenth week was so great that the mortality curve ultimately matched those of the series given the nicotine-epinephrine and the nicotine-mecholyl chloride treatment. This behavior contrasts sharply with that exhibited by the male rats which received injections of solution of nicotine only. These showed a uniform mortality rate throughout the entire course of the experiment and had a considerably lower total mortality than the female rats identically treated, as 10 per

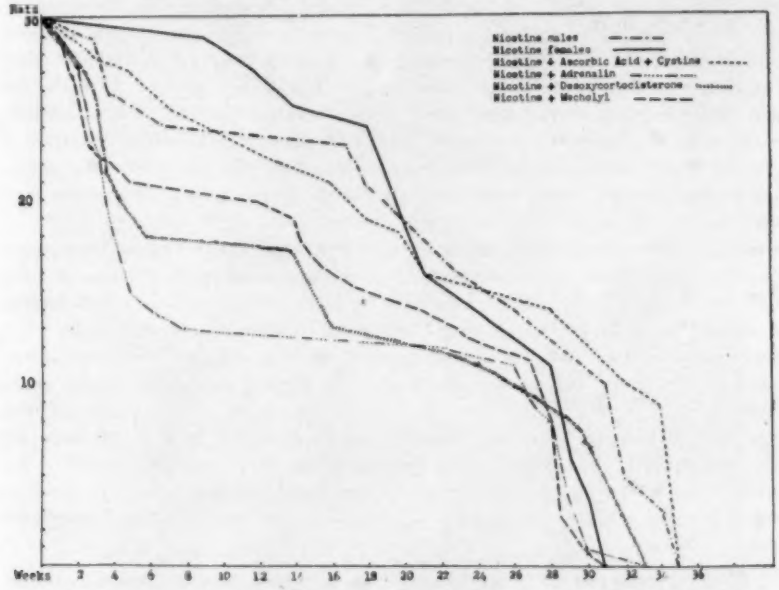

Fig. 1.—Mortality rates of the several experimental series of rats.

cent of the male rats were still alive at the end of the thirty-fifth week. However, by far the lowest mortality rate prevailed among the rats which received cystine and ascorbic acid with their diet. This tendency was particularly manifest during the latter part of the experimental period and was shown by the fact that at the end of the experiment one third of the original number of rats belonging to this series were still alive.

Pathologic Anatomy of Animals Given Nicotine Only.—(*a*) Dogs: Of the 4 dogs which died during the first four weeks of the experiment, 1 had a few hyaline thickenings in the media of the renal arterioles; 3 had marked edema, and 2 a few focal hyalinizations, in the media of the aorta and of the larger elastic arteries. The lungs of these 4 dogs showed extensive purulent and hemorrhagic infiltrations. The adrenal glands showed medullary congestion.

Similar changes in the adrenal glands were found in the 2 dogs which survived for ten months. The thyroid glands of these animals consisted of medium-sized

follicles lined by a single layer of flat epithelial cells and filled with a solid, pink-stained colloid. The anterior lobe of the hypophysis was composed mainly of eosinophilic cells. The myocardial arterioles not infrequently showed thickened and hyaline walls and a proliferated endothelium arranged in palisade formation. Similar lesions were seen in the renal arterioles. The renal artery of 1 dog exhibited a small mushroom-like hyaline thickening of the intima (fig. 2*A*). One dog had similar fibrohyaline intimal deposits in the aorta, while the other dog had a large hyaline scar in the media (fig. 2*B*). In both dogs the inner part of the media of the aorta was edematous. Small scattered perivascular hemorrhages and edema, affecting mainly the vessels at the floor of the fourth ventricle, and some endothelial proliferation of the arterioles were observed in the brain.

(*b*) Rats: In both the male and the female rats the adrenal medulla was congested and consisted of large juicy cells. In a few instances these extended into the cortex. The adrenal medulla of 1 male rat contained a few giant cells with multiple nuclei. The cortex was relatively narrow. A small extracapsular cortical adenoma was found in 1 male rat. These changes were most pronounced in animals which died during the latter part of the experiment. The testes in 7 of 19 male rats studied histologically showed some kind of degenerative lesions. These affected, however, only exceptionally the entire testicular parenchyma, being restricted in general to small, subcapsularly located areas in which the spermatogenic epithelium was degenerated, where spermatid giant cells were present and where some tubular lumens contained calcified necrotic debris.

Some arterioles of the brain, heart, kidneys and lungs showed an edematous and hyaline thickened media, which occasionally contained focal accumulations of large round or oval nuclei (fig. 2*C*). Perivascular hemorrhages and glia cell infiltrations as well as focal degenerations of the nerve substance were found in a few instances in association with the aforementioned vascular lesions. Endothelial proliferations of the arterioles were exceptional. The aorta was always normal except for some medial edema. Subintimal and medial polypous or trabecular calcifications were noted in the pulmonary artery and its branches in 3 of 29 male and female rats studied. The lungs were often congested and contained more or less extensive hemorrhages. Purulent bronchitis and bronchopneumonia were observed in several animals. Marked congestion of the spleen, the liver and the kidneys was a common feature.

Pathologic Anatomy of Rats Given Nicotine plus Other Substances.—(*a*) Nicotine plus Cystine and Ascorbic Acid: Plump processes of medullary tissue extended not infrequently into the cortex from a congested and relatively large adrenal medulla consisting of juicy cells. In 1 instance a small cortical, subcapsularly situated adenoma and two extracapsular cortical adenomas located nearby in the pericapsular fat tissue were found, while in a second case the adrenal medulla contained two small areas of atypical neurogenic tissue composed of large ganglion cells, glia cells, nerve fibrils and a few small cysts (fig. 3*A*). The testes of 10 of the 20 rats studied showed degenerative changes in the spermatogenic epithelium, affecting usually only smaller areas and involving sometimes only one gonad.

Degenerative vascular lesions affecting the arterioles of the brain, lungs, heart and kidneys were represented by hyalinization and thickening of the media and were most frequent in animals that died spontaneously during the latter part of the experiment. A large hyaline thrombus with calcification and fibroblastic invasion at the base was found in the auricle of 1 rat. Whereas similar but less severe vascular changes were present in many of the 9 rats surviving to the end of the experiment,

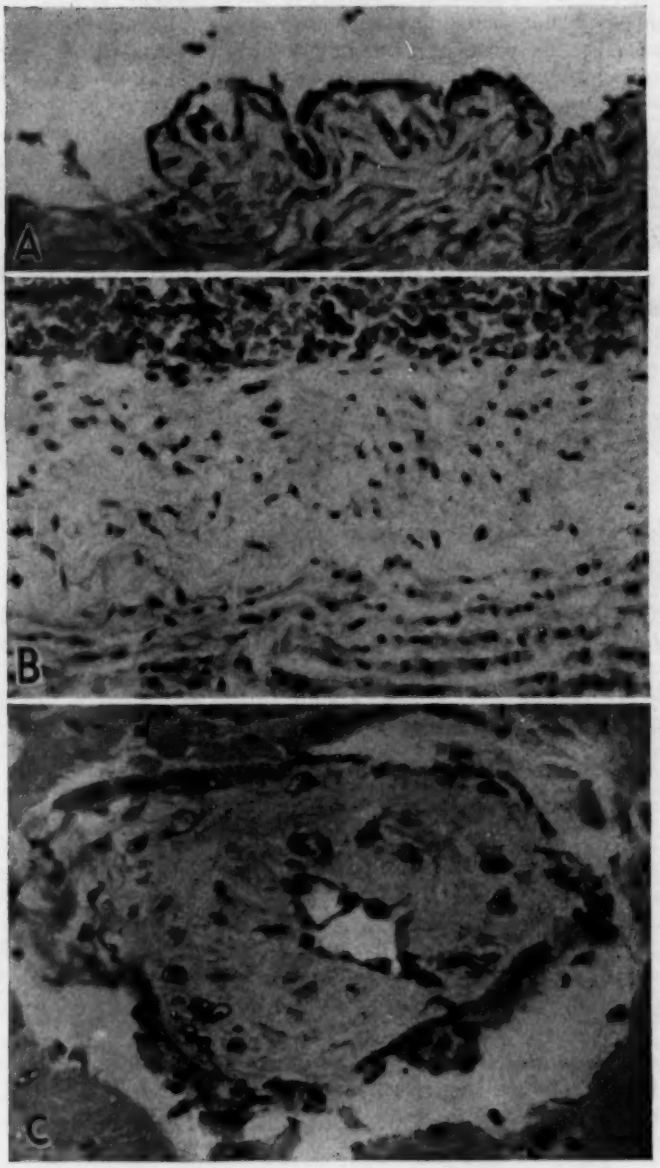

Fig. 2.—*A*, mushroom-like fibrous thickening of the intima in a renal artery. *B*, fibrohyaline intimal thickening of the aorta. *C*, hyalinization of the media of a myocardial arteriole containing swollen round nuclei.

3 of these animals were entirely free from any vascular lesion. Two of the surviving rats exhibited small scars in the renal cortex, which were composed of hyaline glomeruli, interstitial lymphocytic accumulations and cystic tubules. Calcifications in the walls of the pulmonary artery and its branches were found in 10 rats. The internal organs (brain, lungs, liver, spleen, kidneys) often showed hyperemia and edema of a moderate to marked degree. Pulmonary hemorrhages were common.

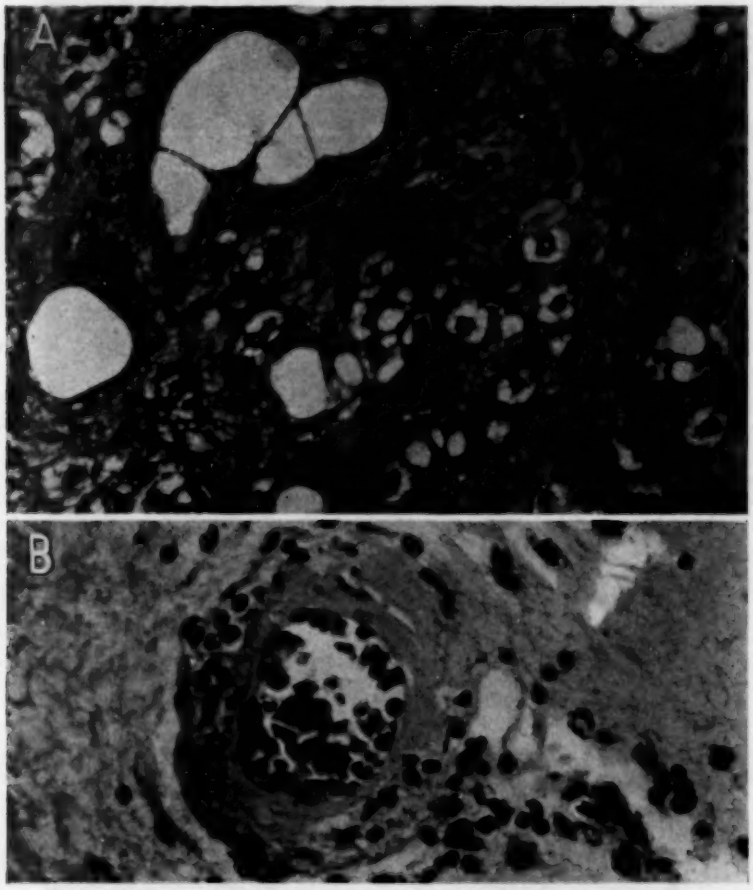

Fig. 3.—*A*, focus of neurogenic tissue in the adrenal medulla, composed of ganglion cells, glia cells and nerve fibrils. *B*, hyalinized and swollen cerebral arteriole with perivascular glia cell infiltration.

(*b*) Nicotine plus Epinephrine: In the rats which died during the first two months of the experiment edema and sometimes hemorrhages in the brain, the lungs, the liver and occasionally the heart were the most striking lesions observed. One rat of this group showed a hemorrhagic erosion of the glandular mucosa of the stomach. Animals which died later usually exhibited adrenal glands with hyperemia and sometimes hyperplasia of the medulla. The medullary cells were large and

juicy. While degenerative and calcifying lesions, occasionally associated with the appearance of spermatid giant cells, were seen in the testes of a few rats, the majority had normal gonads.

Thickened and hyaline arteriolar walls were found in an appreciable number and with increasing frequency in the brain, the lungs, the heart and the kidneys. There was in some vessels focal crowding of hyperchromatic nuclei in the media. Calcifications in the subintima and the media of the pulmonary artery and its branches were seen in only 3 of 22 animals examined histologically. It was noted, on the other hand, that the pulmonary arterioles were not infrequently extraordinarily hypertrophic and showed a swollen and hyaline media. The aorta was normal.

(*c*) Nicotine plus Desoxycorticosterone Acetate: The adrenal glands of those rats which died during the latter part of the experimental period were large and had a hypertrophic, congested or edematous medulla. Hemorrhage into the cortex was present in 1 rat. The testes of 10 rats of the 25 histologically studied animals exhibited various degrees and types of degenerative and necrotic changes. The severity of these lesions increased with the duration of the experiment.

Similar relations were found in regard to the degenerative lesions involving the arterioles of the brain, the heart, the kidneys and rarely the lungs. These lesions consisted chiefly of thickening and hyalinization of the media. Calcifications of the wall of the pulmonary artery and its branches were present in 6 rats. Edema, hyperemia and hemorrhages were noted in the brain, the lungs, the liver and the spleen. The renal glomeruli of several rats exhibited peculiar hydropic or hyaline swelling or albuminous degeneration of the cellular and interstitial structures, with the swollen and pale blue–stained nuclei of the capsule scattered on the surface and within the homogeneous masses.

(*d*) Nicotine plus Mecholyl Chloride: The adrenal glands always showed marked medullary congestion. The medulla was composed of large juicy cells, which in several instances were infiltrating the cortical tissue. Nine of a total of 19 histologically studied rats showed some kind of testicular degeneration. Small atrophic and fibrous areas were present in the pancreas in 2 rats, which were the last ones to die in this series. One of these rats had a mild leukocytic infiltration in the androgenic zone of the adrenal glands.

In a minor number of rats the arterioles of the brain, the heart, the lungs and the kidneys showed some hyalinization and thickening of the media. However, the incidence of these lesions was much lower than that of the previously recorded series. One rat had small calcified foci in the wall of the pulmonary artery just above the base of the heart, while another rat had a serous exudate beneath the aortic intima. In 9 instances the pulmonary artery and its branches contained calcifications in the subintima and the media. Perivascular hemorrhages of the brain were found in 12 rats and were associated in 3 with perivascular gliosis and degenerative foci in the nerve substance (fig. 3 *B*). The lungs, the spleen, the liver and the kidneys were usually considerably congested, and pulmonary hemorrhages were common.

COMMENT

The observations recorded show that, given excessive doses of nicotine over long periods, dogs and rats will have degenerative lesions of the aorta, the large elastic arteries (pulmonary, renal) and the arterioles of the brain, the heart, the lungs and the kidneys. The pathologic changes in the elastic arteries are characterized by hyaline

thickenings of the intima and by fibrosis, hyalinization and calcification of the media. In the arterioles, on the other hand, they consist of hypertrophy, fibrosis and hyaline thickening of the media and the subintima with occasional proliferation of the endothelial lining. It is noteworthy that in the rats the aorta remained in general free from any pathologic changes, while in the dogs it was regularly involved. The vascular responses obtained in dogs and rats through prolonged administration of nicotine are thus similar to those allegedly following excessive exposure to this agent in man and bear a close resemblance to those previously reported in rabbits. The experimental evidence supporting an arteriosclerotogenic action of nicotine is thereby strengthened.

This conclusion receives additional support when proper consideration is given to the morphologic character of the vascular lesions observed. In previous investigations the thesis was advanced that the anatomic type of arteriosclerosis depends on the nature of the causative agent and its particular mechanism of action (Hueper[28]). It was stated that agents which form unstable emulsions with the colloidal solution of the plasma proteins, which are chemically relatively stable and which interfere with the adequate exchange of gases between the interface of blood and vascular wall give rise to the development of atheromatous lesions (cholesterol atheromatosis, polyvinyl alcohol atheromatosis, methyl cellulose atheromatosis, pectin atheromatosis). Disturbances in the quantitative and qualitative relations of the plasma proteins resulting in instability of the colloidal equilibrium of these proteins cause the appearance of fibrohyaline intimal thickenings as well as medial degenerations and calcifications. Increased intravascular pressure as well as vasotonic agents of both the hypertonic and hypotonic types elicit, through the mechanism of ischemic anoxemia or of stagnant anoxemia, fibrohyaline thickenings and intimal proliferations as well as medial degenerations and calcifications. The vascular lesions produced by nicotine in rats and dogs reflect in their morphologic character and their topographic distribution within the vascular tree and vascular wall the vasoconstrictive action of this agent.

It is of interest to note that the toxicity as well as the severity, the number and the distribution of vascular reactions to nicotine could be favorably influenced by exogenous dietary factors (cystine and ascorbic

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acid). However, a relatively high incidence of calcifying vascular lesions of the pulmonary artery and of abnormalities of the adrenal glands was found in this series.

While the addition of the administration of epinephrine to the treatment with nicotine appreciably increased the toxicity of nicotine and thereby accentuated the mortality rate, this management did not result in aggravating significantly the vascular injury as might be expected from the concept of the epinephrinogenic causation of arteriosclerosis in chronic nicotinism. A direct vasculotoxic or indirect cerebral vasotonic action of nicotine gains thus in etiologic importance.

A similar accentuation of the toxic effect of nicotine was apparently attained by the addition of desoxycorticosterone acetate.

The combined treatment of rats with nicotine and mecholyl chloride, two vasculotonic antagonists, resulted, on the other hand, in reduction of the degenerative vascular reactions. The relatively high mortality rate observed among the rats of this series was evidently due to the severe circulatory crises following the injection of both nicotine and mecholyl chloride, evidenced not only by the symptomatic reactions recorded but also by the presence of cerebral hemorrhages.

While the increased susceptibility of female rats to nicotine (Staemmler) is confirmed by these experiments, the contention that there is a chemospecific injurious action on the male gonads (Ehrismann [29]; Hoffstaetter [30]) is not supported. The degenerative testicular changes observed in an appreciable number of rats treated with nicotine seem to result secondarily from the circulatory disturbances induced and are probably causally related to more or less prolonged phases of testicular anoxemia, as hematic or vasoconstrictive hypoxemic agents, such as reduced atmospheric oxygen pressure (sojourn at high altitudes), lead, carbon disulfide and carbon monoxide, elicit functional and anatomic testicular degeneration. It may be emphasized in this connection that neither the testicular nor the vascular lesions can be attributed to "physiologic" old age, as their incidence and severity exceed by far those occasionally observed in a series of 100 normal rats of the same age range.

SUMMARY AND CONCLUSIONS

Rats and dogs given subcutaneous injections of nicotine over eight to ten months were found to have degenerative lesions in the elastic and muscular arteries and arterioles. The morphologic type of arterial changes produced reflects the nature of the mechanism of action of nicotine (vasoconstrictive ischemic anoxemia).

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30. Hoffstaetter, R.: Virchows Arch. f. path. Anat. **244**:182, 1923.

Female rats given injections of nicotine only and male rats given injections of nicotine and epinephrine, nicotine and desoxycorticosterone acetate and nicotine and mecholyl chloride, respectively, showed a much higher mortality rate than male rats receiving nicotine only, particularly male rats given injections of nicotine and kept on a diet containing additions of cystine and ascorbic acid.

Exogenous and, possibly, dietary factors may account in part for the differences in individual susceptibility to nicotine in man and in animals.

The testicular degenerations occurring in an appreciable number of rats treated seemed to be the result of vasoconstrictive episodes of anoxemia.

STUDIES IN VITRO ON THE PHYSIOLOGY OF NORMAL AND OF CANCEROUS CELLS

I. THE EFFECT OF HIGH TEMPERATURE AND OF MOCCASIN VENOM ON THE VIABILITY OF RABBIT LYMPHOCYTES AND POLYMORPHONUCLEAR LEUKOCYTES AS DETERMINED BY THE METHOD OF UNSTAINED CELL COUNTS

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HINES, ILL.

Various types of cells have been extensively investigated from the standpoint of morphology since the days of Virchow. Although the physiology of the cell is as important as its morphology, physiologic studies have been handicapped by the lack of adequate methods. General methods which have been developed for the in vitro study of cell physiology are Warburg's manometric method [1] and tissue culture.[2] Both methods have been found useful but are somewhat difficult to employ in the study of certain physiologic problems.

An attempt was therefore made to find a method which would be as simple as ordinary bacteriologic technic and which could be carried out in any laboratory of pathology. The method that apparently had these qualifications was the counting of the viable cells in a suspension on the basis that imperviousness of a cell to eosin is an indicator of viability. A preliminary report on this procedure was published in 1936.[3]

In the present work the method of unstained cell counts is used to study the effect of elevated temperature and of moccasin venom on the lymphocytes and the polymorphonuclear leukocytes of the rabbit.

METHODS

To obtain suspensions of lymphocytes, the thymus or the spleen was removed from a normal rabbit which had been killed by an intravenous injection of air. The tissue was thoroughly chopped up with sharp small curved scissors. During this process, a small amount of Tyrode's solution was gradually added. The

From the Tumor Research Unit, Veterans Administration.

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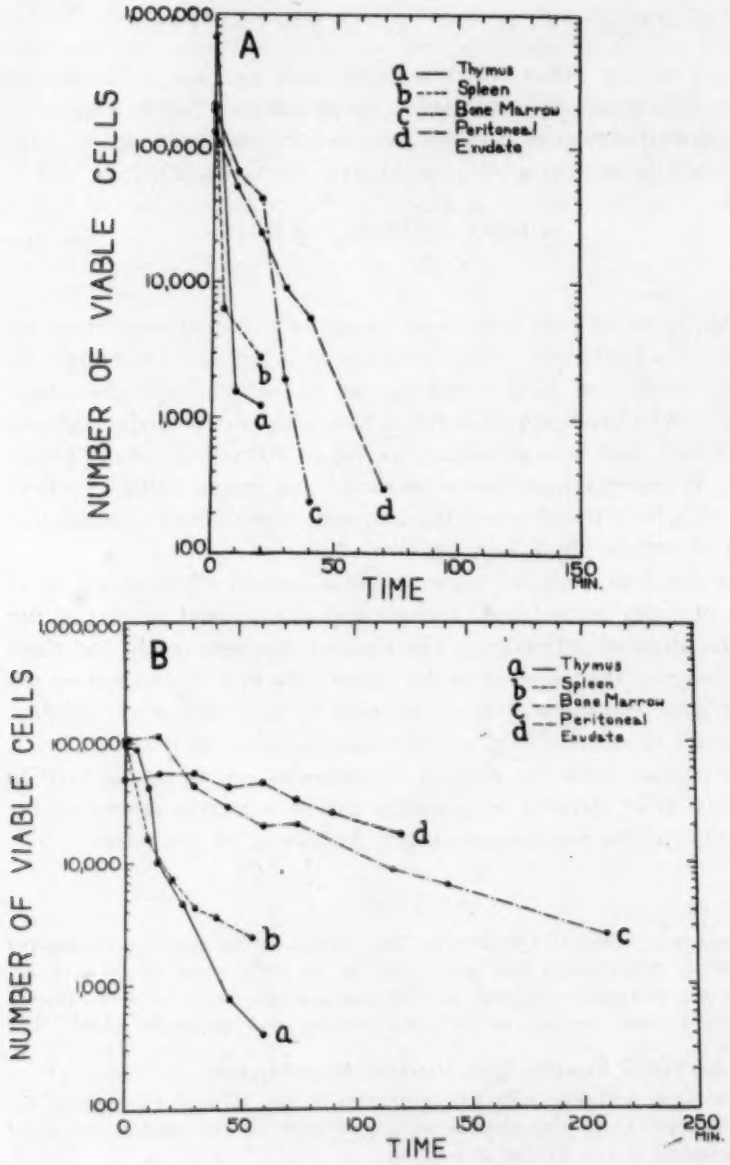

Figure 1

(See legend on opposite page)

minced tissue was filtered in a syringe through 80 gage monel metal wire cloth. On microscopic examination of stained smears, the suspension prepared from the thymus was found to contain isolated cells, most of which were small and large lymphocytes. There were also a few red blood cells. The suspension from the spleen had numerous lymphocytes and very many red blood cells.

The most satisfactory suspension of polymorphonuclear leukocytes was the exudate obtained eighteen hours after an intraperitoneal injection of 200 cc. of a 5 per cent suspension of aleuronat (albuminoid substance and lecithin). The peritoneal exudate thus obtained was centrifuged and the precipitated cells resuspended in a small volume of supernatant fluid, Tyrode's solution or rabbit's serum. Nearly all the cells of the peritoneal exudate were polymorphonuclear leukocytes. A small percentage was large mononuclear cells and red blood cells.

Another suspension of polymorphonuclear leukocytes was obtained from bone marrow. The tissue from the shaft of a rabbit's femur was minced with scissors and filtered through wire cloth. The resulting suspension had different types of cells, but a large percentage was polymorphonuclear leukocytes.

To determine the number of viable cells in these four suspensions, a 1:2,000 solution of eosin in Tyrode's fluid was added (usually 3.8 cc. of dye to 0.2 cc. of suspension). The cells which were stained diffusely red were considered dead, and the cells which were unstained by the eosin were assumed to be viable. The differentiation between the stained and the unstained leukocytes and the red blood cells was nearly always clearcut. The counting of the cells was done in a hemacytometer filled with the suspension-eosin mixture. The results were expressed in cells per cubic millimeter. Most of the counts reported in this paper were performed by technicians.

For the study of the effect of high temperature on cells, the four types of cell suspensions were heated in a water bath maintained at 56, 50 or 45 C. for five to three hundred minutes. The suspensions were contained in small (100 by 13 mm.) stoppered test tubes, each tube having 0.2 cc. of suspension. An eosin solution was added to the heated suspensions, and the stained and unstained cells and red blood cells were counted in a hemacytometer.

EXPLANATION OF FIGURE 1

A, the effect of exposure to heat at 56 C. on the number of viable cells in suspensions derived from rabbit thymus, spleen and bone marrow and in a peritoneal exudate. The ordinates indicate the logarithm of the number of viable cells per cubic millimeter. The complete counts on the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus	691,000	84,000	25,000
Suspension from spleen	204,000	67,000	675,000
Suspension from bone marrow	196,000	34,000	149,000
Peritoneal exudate	130,000	1,000	46,000

B, the effect of exposure to heat at 50 C. on the number of viable cells in suspensions derived from rabbit thymus, spleen and bone marrow and in a peritoneal exudate. The complete counts on the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus	101,000	77,000	6,000
Suspension from spleen	105,000	76,000	390,000
suspension from bone marrow	108,000	17,000	335,000
Peritoneal exudate	51,500	1,500	27,500

Dried moccasin venom was obtained through the aid of Dr. B. W. Carey, of the Lederle Laboratories, Inc. The toxicity of the venom was determined by intradermal injections into rabbits. The minimal intradermal dose was 0.1 cc. of a 1:20,000 dilution, which produced a small reddish black area in twenty minutes.

A mixture of a solution of venom and a cell suspension was incubated at 37 C. in a water bath for various intervals of time; 0.2 cc. of the venom solution was added to 0.2 cc. of cell suspension in a small (100 by 13 mm.) test tube.

After the incubation with venom, the number of viable cells in the cell suspension was again determined. A marked decrease in the number of viable cells as compared with the original count was attributed to the action of venom. Controls were set up to exclude any deleterious effect due to the time of incubation or the Tyrode's solution used.

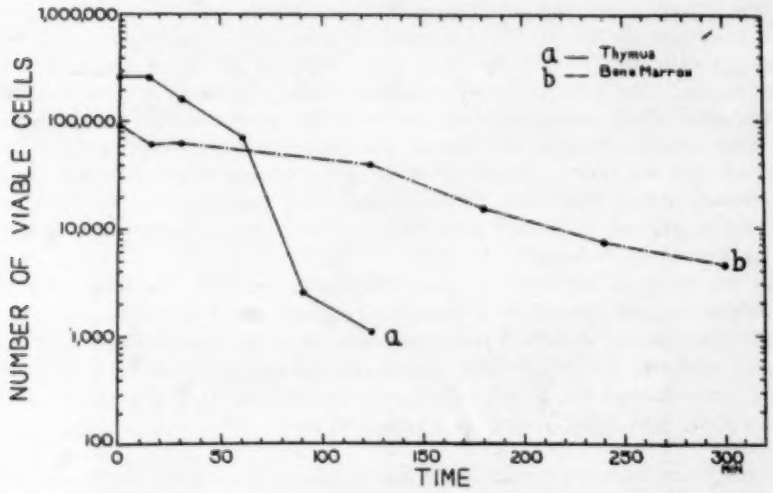

Fig. 2.—The effect of exposure to heat at 45 C. on the number of viable cells in suspensions derived from rabbit thymus and bone marrow. The complete counts on the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus	269,000	350,000	4,000
Suspension from bone marrow	98,000	65,000	147,000

RESULTS

Effect of High Temperature on the Number of Viable Cells.—Suspensions from thymus, spleen and bone marrow and a peritoneal exudate when exposed to temperatures of 56, 50 and 45 C. suffered a decrease in number of viable cells. The results of typical experiments are shown in figures 1 and 2 and are summarized in table 1. The graphs show the rate of decrease in the number of viable cells for each type of suspension and for each temperature studied, the rate of

decrease being represented by the slope of the curve. The table gives the estimated time required to kill 90 per cent of the viable cells of the original suspension.

A and *B* in figure 1 show, in the first place, that the suspensions from the thymus and the spleen were inactivated at approximately the same rate of speed. Ninety per cent of the cells of the two suspensions were killed in less than five or ten minutes at 56 C. and in sixteen minutes at 50 C. (table 1). It seems, then, that the cells in the two suspensions were equally sensitive to the action of the elevated temperature. This finding is consistent with the fact that the nucleated cells of both suspensions are, for the most part, lymphocytes.

It is also seen in *A* and *B* of figure 1 that the rates of inactivation of cells in the peritoneal exudate and the bone marrow suspension were approximately equal. It is estimated that at 56 C., it took twenty-five or twenty-six minutes to kill 90 per cent of the leukocytes in the two

TABLE 1.—*A Comparison of the Effects of High Temperatures on the Cells in a Peritoneal Exudate and in Suspensions of Rabbit Thymus, Spleen and Bone Marrow*

	Minutes Required to Kill 90 per Cent of the Cells			
	Lymphocytes		Polymorphonuclear Leukocytes	
Temperature	Thymic Suspension	Splenic Suspension	Bone Marrow Suspension	Peritoneal Exudate
56 C.	Less than 10	Less than 5	26	25
50 C.	16	16	111	More than 120
45 C.	79	..	226	..

suspensions. It is of interest to note that the mature polymorphonuclear leukocytes of the peritoneal exudate and the immature cells of the marrow had apparently the same rate of death at high temperatures.

The third and most striking point was the difference in the reaction of the suspensions of lymphocytes and polymorphonuclear leukocytes at elevated temperatures. It is seen from figures 1 and 2 that the lymphocytes in the suspensions from the thymus and the spleen were killed at a more rapid rate than the polymorphonuclear leukocytes in the other two suspensions. Table 1 shows, furthermore, that at 56 C. it took less than five or ten minutes to kill 90 per cent of the cells in the thymic and splenic suspensions, compared with twenty-five or twenty-six minutes for the bone marrow suspension and the peritoneal exudate; at 50 C. the corresponding intervals of time were sixteen minutes and two hours; at 45 C. they were seventy-nine and two hundred and twenty-six minutes. It seems, then, that at the three elevated temperatures studied the polymorphonuclear leukocytes of the marrow suspension and the peritoneal exudate were more resistant to the action of heat than the lymphocytes of the thymic and splenic suspensions.

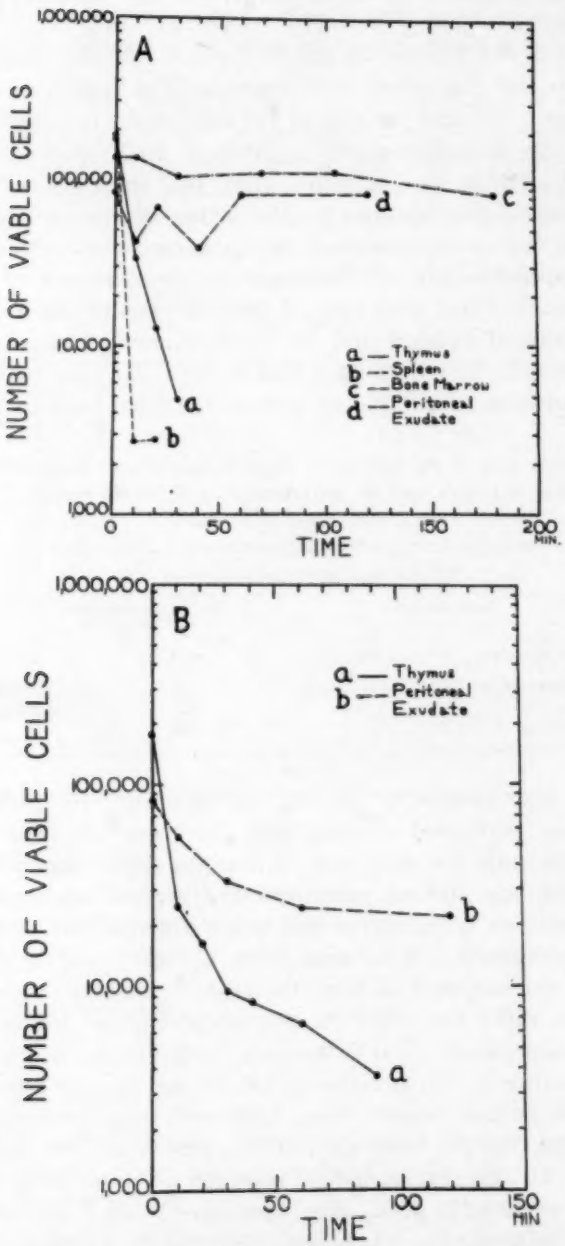

Figure 3

(See legend on opposite page)

Effect of Moccasin Venom on the Number of Viable Cells.—The effect of adding moccasin venom in 1 : 2,000 dilution to an equal volume of a cell suspension is shown graphically in figure 3.

It is seen that the number of viable cells (i. e., those which fail to stain with eosin) in the suspensions from the spleen and the thymus decreased rapidly as a result of the action of moccasin venom. In fact, approximately 90 per cent of the cells were dead (i. e., were capable of staining with eosin) in about ten minutes. Since the cells in both suspensions were largely lymphocytes, it seems that this type of cell is killed rapidly by dilute venom.

Figure 3 *A* also shows the effect of this venom in the same dilution on a peritoneal exudate and on a suspension from bone marrow. In contrast to the previous findings, the venom did not produce any appreciable decrease in the number of viable cells even after one hundred and twenty minutes' incubation. As polymorphonuclear leukocytes were predominant in the bone marrow suspension and the peritoneal exudate, it appears that this type of cell is highly resistant to moccasin venom in 1 : 2,000 dilution.

Further experiments were made to determine the minimal concentration of moccasin venom to which lymphocytes react and the maximal concentration to which polymorphonuclear leukocytes are resistant.

Moccasin venom diluted to 1 : 10,000, 1 : 40,000 and even 1 : 100,000 caused a definite gradual decrease in the number of viable cells in suspensions from the thymus and the spleen. Figure 3 *B* shows the effect of the venom in 1 : 10,000 dilution on a suspension from the thymus. Only 3 per cent of the viable cells persisted after sixty minutes of incubation with venom in this dilution. In contrast, a concentrated solution of

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EXPLANATION OF FIGURE 3

A, the effect of 1 : 2,000 dilution of moccasin venom on the number of viable cells in suspensions derived from rabbit thymus, spleen and bone marrow and in a peritoneal exudate. The complete counts of the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus	178,000	97,000	9,000
Suspension from spleen	188,000	78,000	258,000
Suspension from bone marrow	136,000	31,000	640,000
Peritoneal exudate	85,000	25,000	31,000

B, the effect of 1 : 100 dilution of moccasin venom on the number of viable cells in a peritoneal exudate as contrasted with the effect of 1 : 10,000 dilution of venom on the number of viable cells in a suspension derived from rabbit thymus. The complete counts of the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus	181,000	148,000	5,000
Peritoneal exudate	85,000	25,000	31,000

venom (1:100) caused only a moderate decrease in the number of viable cells of the peritoneal exudate (fig. 3 *B*). After sixty minutes' incubation, 30 per cent of the cells were still viable.

It seems, then, that the lymphocytes of the rabbit are extremely susceptible to the deleterious effects of moccasin venom, but that the polymorphonuclear exudate is almost completely resistant.

Effect of Moccasin Venom on Agglutination and Lysis.—To check the findings by another method, a study was undertaken on the effect of moccasin venom on agglutination and lysis of the lymphocytes and the polymorphonuclear leukocytes of the rabbit.

Equal volumes of a cell suspension and moccasin venom were maintained at 37 C. for one hour. The mixtures were then examined. The formation of firm macroscopic clumps which could not be readily

TABLE 2.—*The Effect of Dilution of Moccasin Venom on the Agglutination and Lysis of the Cells in a Peritoneal Exudate and in a Suspension Derived from Rabbit Thymus*

	Tyrode's Solution	Dilution of Moccasin Venom						
		1:100	1:200	1:400	1:1,000	1:2,000	1:4,000	1:10,000
Suspension from thymus								
Agglutination	0	0	0	0	A	A	A	0
Lysis	0	L	L	L				
Peritoneal exudate								
Agglutination	0	0	0	0	0			
Lysis	0	0	0	0	0			

A = positive macroscopic agglutination.
L = lysis observed on microscopic examination.

broken up was considered as positive agglutination. Lysis was evidenced by absence of intact cells on smears or on examination in a hemacytometer.

The result of a typical experiment is shown in table 2. It is seen that agglutination occurred one hour after the addition of moccasin venom in dilutions of 1:1,000, 1:2,000 and 1:4,000 to a thymic suspension. With venom in higher concentrations there was no agglutination, the suspensions remaining turbid. Microscopic examination showed, however, the absence of intact cells. Evidently the higher concentrations of venom had caused disintegration or lysis of all the cells. It appears, then, that venom produces both agglutination and lysis of the lymphocytes in a suspension of thymus.

A similar experiment was performed with peritoneal exudate (table 2). No definite evidence of either agglutination or lysis of the polymorphonuclear leukocytes was observed, even in high concentrations of moccasin venom (1:100). It seems that the polymorpho-

nuclear leukocytes of peritoneal exudate are markedly resistant to agglutination and lysis by moccasin venom.

Effect of Moccasin Venom on Motility.—The finding of the resistance of the polymorphonuclear leukocyte to moccasin venom is based on indirect methods of determining viability. As the motility of the leukocyte permits direct observation of the viability of the cell, the effect of moccasin venom on the motility of the polymorphonuclear leukocytes was investigated.

Nearly all the cells of a freshly obtained peritoneal exudate were observed to be actively motile and remained so for at least two hours.

Microscopic examination of a mixture of equal volumes of peritoneal exudate and moccasin venom in 1 : 1,000 dilution showed active motility of the cells during the observation period of two hours. Venom in this dilution had no apparent effect on the motility of the cells.

The addition of a concentrated solution of moccasin venom (1 : 100) to the peritoneal exudate caused immediate formation of small microscopic clumps of cells. On incubation the cells wandered away from the clumps and became isolated. These processes were photographed and are shown in figure 4. Figure 4 *A*, taken three minutes after the addition of a 1 : 100 venom solution to a peritoneal exudate, shows a small compact mass. The cells in the periphery are round and do not present any pseudopods. Nine minutes later (fig. 4 *B*) the peripheral cells are elongated and irregular in shape and have moved a short distance from the clump. In twenty-seven minutes after the beginning of the experiment (fig. 4 *C*) all the cells of the clump are isolated from each other, are irregular in shape and have one or more pseudopods.

Further study showed that the cells treated with the 1 : 100 venom solution were not as active as the cells in control preparations. The treated cells had sluggish motility for thirty minutes. A few of the cells retained that motility for as long as two hours. The high concentration of venom had some injurious effect on the cells, but many of the polymorphonuclear leukocytes remained viable for at least thirty minutes. This finding is in agreement with that obtained by the method of unstained cell counts (fig. 3 *B*).

The work just described was controlled by study of a suspension from the thymus under identical conditions. Venom in dilutions of 1 : 1,000 and 1 : 100 caused the lymphocytes in this suspension to disintegrate within sixty minutes, leaving a small amount of amorphous granular debris.

These experiments on motility showed conclusively that moccasin venom in 1 : 1,000 dilution killed and lysed lymphocytes rapidly but had no effect on the motility and viability of polymorphonuclear leukocytes.

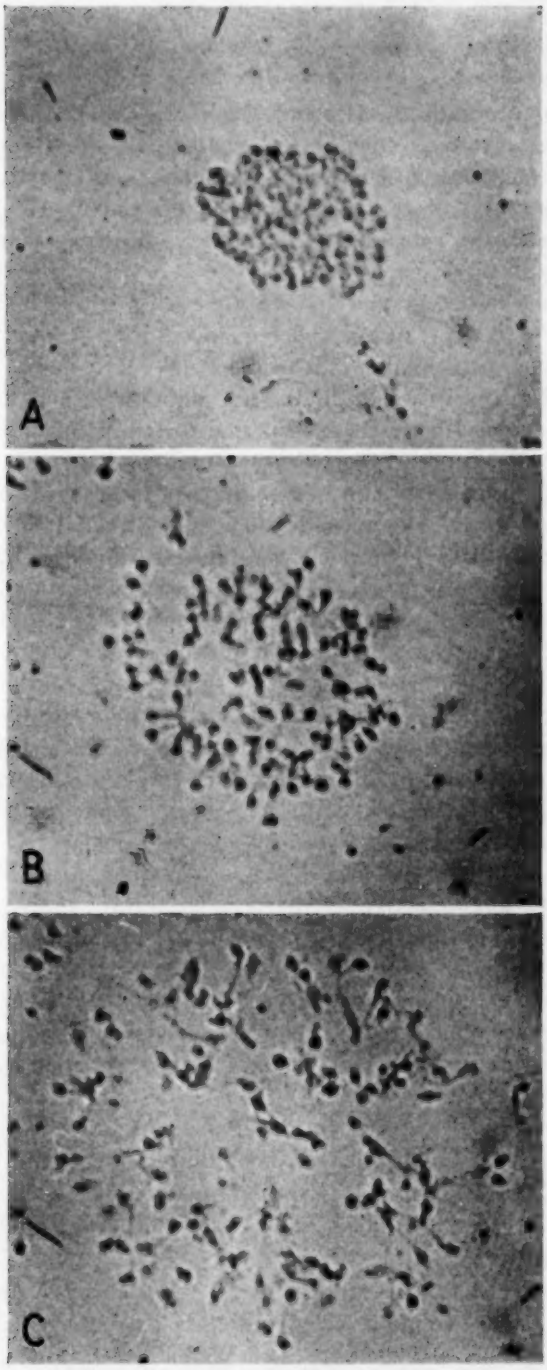

Fig. 4.—The effect of moccasin venom (1 : 100) on the motility of polymorphonuclear leukocytes in a peritoneal exudate (*A*) three minutes, (*B*) twelve minutes and (*C*) twenty-seven minutes after the addition of the venom.

COMMENT

Imperviousness to Eosin as a Measure of Cell Viability.—What is meant by the death of a cell? How can one differentiate between a dead and a viable cell?

A viable cell may be defined as one having certain essential physiologic functions. Two essential properties, which are probably common to all types of living cells, are known, namely, (1) respiration and (2) selective permeability or impermeability of the cell wall to certain substances. These essential properties should be differentiated from the specialized functions which are found only in certain types of cells. Such specialized functions include (1) mitotic and amitotic division, (2) motility, (3) phagocytosis and (4) contractibility. A cell that has lost one or more of the specialized functions is not necessarily dead. However, a cell that has lost either of the two essential properties, respiration or selective permeability, may be considered dead.

Selective permeability can be determined by means of a suitable dye. Supravital dyes have been used to test for difference in permeability or stainability of dead and living cells. Trypan blue was used by Pappenheimer [4] and neutral red by Achard [5] and Belkin and Shear.[6] It is necessary to use a dye which is water soluble and nontoxic, which gives good differentiation between stained and unstained cells and which rapidly stains cells known to be dead. Eosin seems to satisfy these criteria and has been used in this work.

Viable cells are probably impervious to eosin. A cell which is stained by eosin has lost this impermeability and is in all probability dead. The converse proposition is not, however, necessarily true. It cannot be stated so definitely that a cell which resists staining with eosin is viable. There may be conditions in which the cell loses its property of respiration but retains its resistance or impermeability to eosin. It is also possible that certain chemicals may inhibit the staining of dead cells by eosin. It may, therefore, be useful to apply other tests of viability under certain conditions.

In this work it has been assumed that a cell which takes the eosin stain is dead and one which resists the stain is viable.

Effect of High Temperature on the Viability of Cells.—Several investigators have studied the effect of heat on the viability of cells. Clowes [7] showed that a tumor of mice lost its property of transplantability when the cells were exposed to a temperature of 45 C. Pincus and Fischer [8] found that chicken osteoblasts in tissue culture were killed after a six minute exposure to a temperature of 50 C.

4. Pappenheimer, A. M.: J. Exper. Med. **25**:633, 1917.
5. Achard, C.: Brit. M. J. **2**:1416, 1910.
6. Belkin, M., and Shear, M. J.: Am. J. Cancer **29**:483, 1937.
7. Clowes, G. H. A.: Brit. M. J. **2**:1548, 1906.
8. Pincus, G., and Fischer, A.: J. Exper. Med. **54**:323, 1931.

In the present study, it took sixteen minutes' exposure to a temperature of 50 C. to kill 90 per cent of the lymphocytes in the thymic suspension and it took more than one hundred and twenty minutes to kill the polymorphonuclear leukocytes in the peritoneal exudate. These results are not directly comparable with those obtained by Clowes and Pincus and Fischer because of the differences in the cells studied and in the methods used for determining viability.

According to the method of unstained cell counts, it appeared that the polymorphonuclear leukocyte is more resistant to heat than the lymphocyte. Experimental factors that may be responsible for this observation are differences in the eosin stainability of heated lymphocytes and polymorphonuclear leukocytes or variations in the milieu of the cells in suspension. It is, however, probable that the observed greater resistance of the polymorphonuclear leukocyte as compared with the lymphocyte is due to morphologic and physiologic differences within the cells.

Susceptibility of Rabbit Lymphocytes and Resistance of Polymorphonuclear Leukocytes to Moccasin Venom.—Some toxins are known to act on specific types of cells. Tetanus and botulinus toxins, for example, act on the central nervous system, and tetanolysin and streptolysin on the red blood cells.

In contrast, moccasin venom is reputed to act on many, if not all, types of cells. Flexner and Noguchi [9] showed that moccasin venom causes lysis of the cells of kidney, liver, testis and tracheal epithelium in suspension.

The method of unstained cell counts permitted reexamination of the effects of this venom on lymphocytes and polymorphonuclear leukocytes of the rabbit. The polymorphonuclear leukocytes were found to be highly resistant to the lethal, agglutinative and lytic action of moccasin venom. The resistance of these cells was in marked contrast to the susceptibility of the lymphocytes. The difference in the reaction of the two types of cells is under further investigation.

SUMMARY

According to the method of unstained cell counts, the polymorphonuclear leukocytes of the rabbit survive a longer period than the lymphocytes at the elevated temperatures of 56, 50 and 45 C.

Moccasin venom has the capacity of killing, agglutinating and lysing the lymphocytes but has little or no effect on the viability and motility of the polymorphonuclear leukocytes.

9. Flexner, S., and Noguchi, H.: J. Path. & Bact. **10**:111, 1905.

See vol. 36, p 126 for explanation of corrections for fig. 2 + 3.

PINEALOMA

A CLINICOPATHOLOGIC STUDY OF SEVEN CASES WITH A REVIEW OF THE LITERATURE

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The term "pinealoma" was first suggested by Krabbe,[1] in 1923, for the primary tumors of the pineal body representing neoplasia of pineal tissue. Pinealoma characteristically consists of two types of cells that in many instances show a type of arrangement suggestive of the mosaic pattern observed in pineal tissue at the time of birth. Not all the primary tumors of the pineal body may be classified as pinealoma, however, for Bing, Globus and Simon,[2] in 1938, collected 177 cases of pineal tumor from the literature including, besides those of pinealoma, instances of several types of glioma, instances of a teratomatous type and many cases of tumor not histologically verified. Most of the previous studies of this subject (Horrax and Bailey[3]; Globus and Silbert[4]; Dandy[5]; Baggenstoss and Love[6]) have been concerned mainly with pineal tumors in general and the clinical symptoms produced, because a significant number of pineal tumors in preadolescent boys have been associated with precocious puberty. This has raised the question whether the pineal body is not an endocrine gland. There has been no attempt, however, to collect or to study the tumors with the designation of pinealoma as a group. If the pineal body has a function and its tumor is concerned with the production of precocious puberty, such a study should be of prime importance. Noteworthy histologic contributions to the knowledge of pinealoma have been made by Horrax and Bailey[3] and by Globus and Silbert,[4] although these two groups of investigators do not agree on several fundamental points.

The present report is a clinicopathologic study of 7 cases of pinealoma selected from 14 cases of primary tumor of the pineal body that have been observed in the neurosurgical services of the Barnes Hospital and the St. Louis Children's Hospital during the past twenty years. A pathologic study of tumors diagnosed as pinealoma appeared worth while in the light of the differing opinions because the collection of 7 cases of this rare type of tumor provides a sufficiently large number to allow an adequately detailed clinical and pathologic study. In order to broaden the study and further evaluate the problem of precocious puberty associated with pineal tumors, the previously reported cases of pinealoma have been collected and reviewed.

REPORT OF CASES

CASE 1.—J. B., a white man aged 24, was admitted to the Barnes Hospital Dec. 26, 1939. He had suffered from double vision for six months, and recently this had been accompanied by headaches, nausea and vomiting, and generalized weakness.

From the Departments of Pathology (Dr. Russell) and Neurosurgery (Dr. Sachs), Washington University School of Medicine.

1. Krabbe, K. H.: Endocrinology **7**:379, 1923.
2. Bing, J. F.; Globus, J. H., and Simon, H.: J. Mt. Sinai Hosp. **6**:935, 1938.
3. Horrax, G., and Bailey, P.: Arch. Neurol. & Psychiat. **13**:423, 1925.
4. Globus, J. H., and Silbert, S.: Arch. Neurol. & Psychiat. **25**:937, 1931.
5. Dandy, W. E.: Arch. Surg. **33**:19, 1936.
6. Baggenstoss, A. H., and Love, J. G.: Arch. Neurol. & Psychiat. **41**:1187, 1939.

Examination disclosed diplopia, with the left eye turning in slightly, limitation of upward gaze and bilateral papilledema. A ventriculogram obtained Jan. 2, 1940 revealed that the lateral ventricles were moderately dilated. It was felt that the patient had a tumor in the posterior fossa, and cerebellar craniotomy was done. Because of the poor condition of the patient, the operation was terminated before the fourth ventricle could be explored. Following the operation, his condition became progressively worse, and he died January 3.

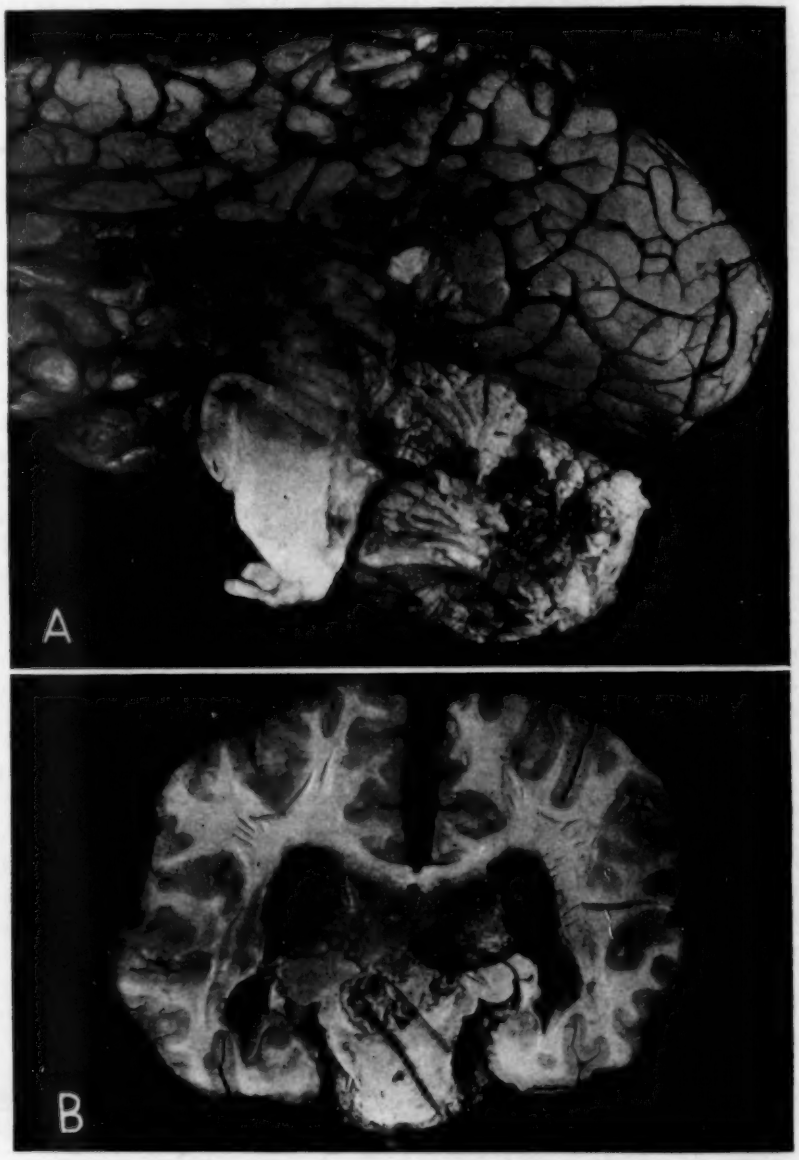

Fig. 1.—Gross appearance of pinealoma in 2 cases: *A* (case 1), sagittal section through the brain. The tumor mass is seen beneath the splenium of the corpus callosum lying on the corpora quadrigemina. It is sharply demarcated from the adjacent brain tissue. *B* (case 2), coronal section taken through the pons and the pulvinar of the thalamus. The tumor completely fills the space beneath the corpus callosum and the interbrain structures. Note the tumor tissue lining both lateral ventricles.

In reviewing the ventriculograms it is obvious that there was no air in the posterior part of the third ventricle, and the correct localization should have been made, and the pineal region should have been directly attacked via a cerebral flap.

Necropsy (seven hours after death; restricted to the examination of the head).—The body was well developed and well nourished. The head had been recently shaved, and there was a fresh craniotomy wound. The brain weighed 1,370 Gm. and was removed without difficulty. There was a small amount of subarachnoidal hemorrhage over the cerebellum. A sagittal section through the brain revealed a mass, 1.2 by 2.3 by 1.8 cm., attached to the habenular commissure in the roof of the third ventricle beneath the splenium of the corpus callosum. The tumor was encapsulated and projected posteriorly over the corpora quadrigemina (fig. 1 *A*). A moderate degree of internal hydrocephalus was present.

Histologic Examination.—A section taken from the tumor revealed a moderately cellular tissue composed of large cells with intermingled small cells showing no characteristic arrangement. A moderately abundant connective tissue stroma was scattered diffusely throughout the tumor. The large cells showed marked pleomorphism and no consistently characteristic shape. Some were nearly round with scant cytoplasm and measured up to 30 microns in diameter, while others were elongated, with abundant cytoplasm. The nuclei of the large cells were usually round or vesiculated with a prominent nucleolus and the chromatin heavily concentrated at the nuclear membrane. Mitotic figures were occasionally observed in the large cells. The small cells were indistinguishable from lymphocytes. They showed moderate pleomorphism and varied in size from one closely resembling a large lymphocyte with a moderate amount of basophilic staining cytoplasm and a nucleus with reticulated chromatin to one resembling a small lymphocyte with scant cytoplasm and a deeply chromatic nucleus. No mitotic figures were observed in the small cells. A phosphotungstic acid–hematoxylin stain revealed a moderate number of deep blue–staining intercellular fibrils between the large cells, some coarse and some fine (fig. 3 *C*).

Case 2.—W. R., a white boy aged 17, entered the Barnes Hospital June 17, 1923. He had suffered from a cerebrospinal rhinorrhea for several months following an operation for sinusitis, which was thought to be the cause of his severe headaches. On entry he showed the signs and symptoms of meningitis, and in spite of treatment he died of meningitis ten days later. Craniotomy was not performed since the cause of his headaches was not suspected.

Necropsy (two hours after death; significant changes occurred only in the brain).—The convolutions were flattened, the sulci were obliterated, and the subarachnoidal space was filled with a heavy, grayish yellow exudate. A finely granular, grayish pink tumor filled the space beneath the splenium of the corpus callosum and the third ventricle. The pineal body was identified in the center of this mass by small areas of calcification. The same type of tissue was observed in the third ventricle and lining the walls of both lateral ventricles (fig. 1 *B*). In some areas the tumor lining the ventricles measured 1 cm. in thickness. A moderate degree of internal hydrocephalus was present.

Histologic Examination.—All sections of the tumor showed a moderately cellular tissue, the cells of which were of two distinct types. One was a large oval or round cell that showed only slight pleomorphism. The cells of this type tended to form closely packed groups, and these groups were surrounded by cells of the second type, which was a much smaller cell resembling a lymphocyte. In a few areas, however, cells of the two types were indiscriminately intermingled. The small cells showed slight pleomorphism and were enmeshed in a moderate amount of connective tissue stroma containing blood vessels. Mitotic figures were observed frequently in the large cells but never in the small cells. With phosphotungstic acid–hematoxylin staining a few extracellular blue-staining fibrils were noted between the large cells in the areas of the tumor where cells of the two types were indiscriminately mixed.

Case 3.—F. C., a white boy aged 17, was admitted to the Barnes Hospital Sept. 21, 1931. He had suffered severely from prostrating headaches for three months, and recently these had been accompanied with vomiting, staggering gait and double vision. The patient's family noticed that he had become emotionally unstable and that a masklike countenance had developed. The boy was well developed, with slow speech. There was marked inequality of the pupils, with the left larger than the right, and neither reacted to light but both responded to accommodation. Both optic disks were choked, with considerable exudate. Both sixth cranial nerves were paralyzed, and upward gaze was definitely impaired. The facial nerve on the left was paralyzed. Pathologic toe signs were present on the left.

The diagnosis seemed so obvious that cerebellar craniotomy was done September 24. The exploration disclosed no tumor or other abnormality. There was no improvement, and the neurologic signs increased in severity. Therefore, November 6, a ventriculogram was made, which showed clearly that we were dealing with a tumor in the posterior portion of the third ventricle. Occipital craniotomy was performed. This time 2 Gm. of soft, grayish red tumor

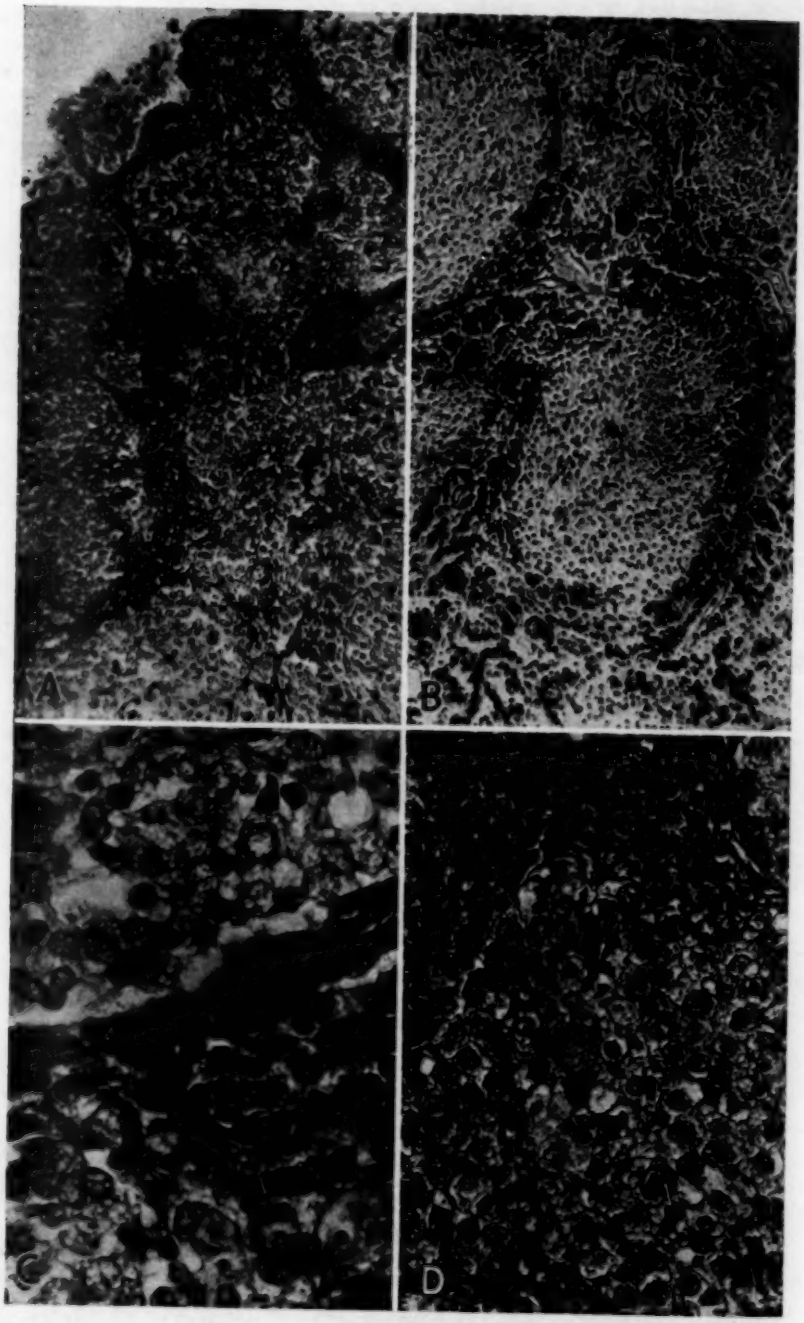

Figure 2

(See legend on opposite page)

tissue was removed from the region of the pineal body. The patient never regained consciousness and died six hours after the operation.

Necropsy (twelve hours after death; only pertinent observations are noted).—The brain was removed with some difficulty due to the adhesions in the posterior fossa from the previous operation. A soft, light pinkish gray tumor tissue filled the space between the splenium of the corpus callosum and the corpora quadrigemina. The tumor infiltrated the superior and inferior colliculi and the brachium conjunctivum. The lateral ventricles were moderately dilated and contained several clots of blood.

Histologic Examination.—All sections of tumor removed at operation and at necropsy disclosed a moderately cellular tissue, the cells of which were of two types. One type was a large cell, which showed a moderate amount of pleomorphism. In some instances it was irregularly outlined and had abundant, moderately basophilic cytoplasm with a round or slightly indented chromatic nucleus. In other instances, however, it was almost round or polygonal, with cytoplasm taking a less basophilic stain. The second type was a small cell showing moderate pleomorphism and resembling a lymphocyte, with scant basophilic cytoplasm and a deeply chromatic nucleus. In some instances it resembled a small lymphocyte, while in others it resembled a large lymphocyte. Generally the large and the small cells were indiscriminately intermingled, but in a few areas of the tumor small collections of from ten to twenty-five large cells were surrounded with broad zones of the small cells, the arrangement suggesting a mosaic pattern. Connective tissue stroma in moderate amount with small blood vessels was diffusely scattered throughout the tumor. Mitotic figures were observed frequently in the large cells but never in the small cells. A phosphotungstic acid–hematoxylin stain revealed a moderate number of deep blue–staining intercellular fibrils between the large cells.

Case 4.—W. H., a white boy aged 14, was admitted to the St. Louis Children's Hospital Sept. 3, 1929. Beginning five months before entry he had complained of severe headaches, which had later been associated with vomiting, convulsions and periods of profound stupor. The boy was stuporous and responded poorly. The physical, sexual and mental development was in no way unusual. There was horizontal nystagmus to the right, with conjugate deviation of the eyes to the left and choking of the disks. He had several convulsions after entry, and a ventriculogram indicated a tumor in the region of the third ventricle. Transcallosal cerebral craniotomy disclosed a vascular tumor in the region of the pineal body, and 3 Gm. of tumor tissue was removed. The patient died shortly after the operation.

Necropsy (five hours after death, limited to the head).—The brain was easily removed, and there was a moderate amount of subarachnoidal hemorrhage over both occipital lobes and the cerebellum. The corpus callosum, the vermis and the corpora quadrigemina showed several irregularly outlined operative defects containing small amounts of clotted blood. No tumor tissue was identified, and several microscopic sections taken from these areas subsequently showed no tumor. The tumor had been completely removed at operation. There was an advanced degree of internal hydrocephalus.

Histologic Examination.—Several sections of the tumor removed at operation disclosed highly cellular tissue, the cells of which were of two types. One type was a large round or polygonal cell showing no pleomorphism, with clear, slightly reticulated cytoplasm that in many instances formed a clear halo around the nucleus. The nucleus of the large cell was round and moderately chromatic, and usually contained a prominent nucleolus. In some areas of the tumor only the large cells were observed, and in these areas there was only a minimal amount of connective tissue stroma with small blood vessels. In other parts of the

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Explanation of Figure

Microscopic details of pinealoma are shown.

A (case 5), photomicrograph taken from an area showing only the large cells. These cells resemble the type cell seen in oligodendroglioma. They are round or polygonal and have clear cytoplasm giving a clear halo around the nucleus. Note a prominent nucleolus in the nucleus of the cells. Hematoxylin and eosin stain; × 570.

B (case 5), section of the tumor showing mostly small cells. One large cell is seen in the center of the field. The small cells vary in size from a cell resembling a small lymphocyte to a cell resembling a large lymphocyte. Hematoxylin and eosin stain; × 1,100.

C (case 1), small and large pineal cells indiscriminately mixed. Coarse and fine intercellular fibrils are seen in all parts of the field. Phosphotungstic acid–hematoxylin stain; × 570.

D (case 5), blepharoplastic granules in the cytoplasm of the large cells. They are seen as small black dots. These granules are stained blue with phosphotungstic acid–hematoxylin stain. Phosphotungstic acid–hematoxylin stain; × 900.

tumor, however, the second type of cell was seen as a small round cell resembling a small or a large lymphocyte. In some areas only the small cells were noted, but in other areas they surrounded collections of the large cells to form a mosaic pattern. The small cells were always accompanied by a moderately abundant connective tissue stroma containing blood vessels. Numerous mitotic figures were noted in the large cells but never in the small cells. A phosphotungstic acid–hematoxylin stain revealed scattered blue-staining blepharoplastic granules in the cyptoplasm of the large cells.

CASE 5.—E. M., a white girl aged 10, was admitted to the St. Louis Children's Hospital Dec. 17, 1929. For five months before entry her vision had been only perception of light, and for two months she had suffered from periodic headaches, many of which were accompanied by nausea and vomiting. The child's parents had noticed that she drank unusually large amounts of water and urinated frequently. The general physical examination revealed no abnormality, and the child's physical, mental and sexual development was regarded as normal. Neurologic examination disclosed bilateral optic atrophy, marked weakness of the left internal rectus muscle, diminution of the activity of the deep reflexes, with both ankle jerks absent, and bilateral pathologic toe signs.

Because of the optic atrophy and roentgen studies of the skull which showed slight enlargement of the sella turcica, a pituitary tumor was suspected. Frontal craniotomy was performed December 23. A ventricular puncture disclosed cerebrospinal fluid under increased presssure and a moderate degree of internal hydrocephalus. A solid tumor was seen in the region of the optic chiasm; because of its close proximity to the carotid artery and the optic chiasm, it was only partially removed. The patient had an uneventful convalescence and was improved when discharged from the hospital Jan. 9, 1930.

She was readmitted to the hospital June 3. The history obtained from the parents at that time disclosed that she had recovered a moderate amount of vision following the first operation but that during the three weeks before entry she complained of painful urination and generalized weakness of the legs. The weakness of her legs became so profound that she was confined to bed for the two weeks prior to admission. There was spastic paraplegia with a definite sensory level corresponding to the ninth dorsal segment. The knee and ankle jerks were hyperactive, and there were bilateral pathologic toe signs. June 6 laminectomy was performed and soft reddish tissue removed from around the spinal cord at about the fifth dorsal segment. Convalescence was satisfactory, but there was only slight return of sensation in the lower extremities with no return of motor power. High voltage roentgen radiation was given over the lesion. The patient was discharged from the hospital unimproved July 3.

Specific details of the patient's course after her discharge from the hospital are not known except that she died from her disease in another hospital several months later.

Histologic Examination.—Sections from the tumor removed at the first and at the second operation all revealed the same type of growth. The tumor was composed of masses of large round or polyhedral cells intermingled with cells of a second type resembling lymphocytes. In some instances the small cells surrounded large groups of closely packed large cells, forming a mosaic pattern. The large cells showed no pleomorphism and had a moderate amount of slightly reticulated clear cytoplasm that in many instances gave a clear halo surrounding the round chromatic nucleus. In many areas only the large cells were found (fig. 2 *A*), and the diagnosis of oligodendroglioma would have been strongly considered had only these areas been examined, for these large cells strikingly resembled the type cell of oligodendroglioma. The finding of small cells in other parts of the section and a mosaic pattern (fig. 3 *D*) plus the fact that the large pineal cells frequently contained blepharoplastic granules (fig. 2 *D*), which are never seen in oligodendroglial cells, were important differential diagnostic points. The small cells resembled lymphocytes and showed gradations in size from a form resembling a large lymphocyte to a form resembling a small lymphocyte (fig. 3 *B*). Mitotic figures were frequently observed in the large cells but were never observed in the small ones. A phosphotungstic acid–hematoxylin stain of the tumor disclosed a moderate number of blue-staining blepharoplastic granules within the large cells.

CASE 6.—R. T., a Negro boy aged 9, was admitted to the St. Louis Children's Hospital Dec. 5, 1939. He had suffered from attacks of severe headache for four months and had vomited severely on several occasions. He was well developed, alert and cooperative, with no abnormal physical, mental or sexual development. The significant observations were bilateral unsustained ankle clonus, dilated pupils, choked disks and slight bilateral exophthalmos. December 12 a ventriculogram was obtained which was interpreted as indicating a lesion of the posterior fossa. Cerebellar craniotomy was performed, but no tumor was found. In reviewing the plates now it is clearly evident that there was a lesion in the posterior part of the third ventricle, as no air filled this region. Cerebellar craniotomy was performed

again December 29, because the patient's condition had become progressively worse. At this time 14 Gm. of tumor tissue lying anterior to the cerebellum in the region of the corpora quadrigemina was removed by sucker. Following the operation, the patient had marked respiratory difficulty and was kept in an oxygen tent for several weeks but died Feb. 21, 1940.

Necropsy (twenty hours post mortem).—Except for advanced bronchopneumonia in the lower lobes of the lungs the significant observations were in the brain. This was removed with considerable difficulty due to fibrous adhesions from the two operations. The weight of the brain was normal, 1,100 Gm. Operative defects were observed on the vermis, the colliculi and the floor of the fourth ventricle. A small piece of grayish pink tissue, measuring 1.5 by 1.3 by 0.8 cm., was attached to the tentorium. The third and the lateral ventricles were moderately dilated.

Histologic Examination.—Sections of the tumor removed at operation and the nodule of tumor attached to the tentorium at necropsy all disclosed a highly cellular tissue, the cells of which were of two types. One type was a large round or polygonal cell that had abundant clear cytoplasm and a round, moderately chromatic nucleus. The cells of this type showed little or no pleomorphism and were closely packed into large groups which in turn were

TABLE 1.—*Summary of the Pertinent Pathologic Observations in the Seven Cases Reported*

					Large Cells				Small Cells		
No.	Age, Yr.	Sex	Location of Tumor	Hydrocephalus	Pleomorphism	Mitotic Figures	Blepharoplasts	Intercellular Fibrils	Pleomorphism	Mitotic Figures	Mosaic Pattern
1	24	M	Pineal region	Moderate	+++	+	0	++	++	0	0
2	17	M	Pineal region, corpora quadrigemina, lateral ventricle	Moderate	+	++	0	+	+	0	++
3	17	M	Pineal region, corpora quadrigemina	Moderate	++	+++	0	+	++	0	+
4	14	M	Pineal region	Advanced	0	+++	+	0	++	0	++
5	10	F	Pineal region	Moderate (determined at operation)	0	++	++	0	++	0	+
6	9	M	Pineal region, extension to tentorium	Moderate	0	+++	+++	0	++	0	++
7	2	F	Pineal region	Advanced (determined at operation)	0	0	0	0	+	0	++++

surrounded by cells of the second type, this arrangement giving the effect of a mosaic pattern. The second type of cell was a small round cell resembling a lymphocyte with a small amount of basophilic cytoplasm and a round, slightly oval or indented, deeply chromatic nucleus. The small cells showed variations in size and shape from cells closely resembling large lymphocytes to cells resembling small lymphocytes. Mitotic figures were frequently observed in the large cells but never in the small cells. Many blue-staining blepharoplastic granules were seen within the cytoplasm of the large cells in a phosphotungstic acid–hematoxylin stain.

CASE 7.—C. S., a white girl aged 2, was admitted to the St. Louis Children's Hospital May 24, 1927. For two months before entry her parents had noticed that she frequently stumbled and occasionally fell. During this time there had been periodic attacks of vomiting. There was no abnormal physical, mental or sexual development. Neurologic examination revealed marked incoordination of the movements of the extremities, bilateral pathologic toe signs, bilateral sustained ankle clonus, bilateral papilledema and dilatation of pupils, which did not react to light. May 27 cerebellar craniotomy disclosed that the lateral ventricles were markedly dilated, and 12 Gm. of grayish pink tumor tissue was removed from the region of the pineal body by splitting the vermis. The patient made an uneventful recovery and was discharged from the hospital July 17 in good condition. Further attempts to observe the patient were unsuccessful, and it is not known whether the operation effected a complete cure or not.

Histologic Examination.—Sections of the tumor disclosed tissue composed of cells of two types. One type was a large cell having an ill defined cell membrane with clear cytoplasm and a small hyperchromatic nucleus. These cells were closely packed into large masses but showed no appreciable pleomorphism. The second type of cell was a small cell resembling a large lymphocyte. The cytoplasm was basophilic, and the nucleus contained abundant deeply basophilic chromatin that in some instances was slightly reticulated. These cells were grouped together in broad zones and surrounded collections of the large cells. This unusual arrangement of the two types of cells gave to the section when examined under low power magnification the appearance of a mosaic pattern (fig. 2 *B*). A phosphotungstic acid-hematoxylin stain of the tumor revealed no intercellular or intracellular fibrils or blepharoplastic granules. There was discernible in this stain, however, a connective tissue stroma accompanying the collections of the small cells, with a moderate number of small blood vessels. No connective tissue stroma or blood vessels were observed in the collections of the large cells.

The pertinent gross and histologic observations made in the 7 cases are summarized in table 1.

HISTOGENESIS OF THE TWO TYPES OF CELLS IN PINEALOMA

Our knowledge of the histogenesis of the two types of cells observed in pinealoma is derived from studies of the development of the pineal body in man. The anlage for the pineal body appears in man during the second month of fetal life with hyperplasia of the ependymal cells in the posterior part of the roof of the diencephalon. Coincident with the hyperplasia and the initial piling up of the primitive ependymal cells there appears a small evagination of the wall of the third ventricle into the cell mass to form a small diverticulum. According to Krabbe,[7] this diverticulum, by splitting the cell mass into an anterior and a posterior part, presumably forms two separate anlages, the anterior one representing the parapineal organ and the posterior one the pineal organ.

In the fourth month of fetal life the anterior and posterior anlages increase in size, with the result that there is marked narrowing of the cavity of the pineal diverticulum. Fusion of the anterior and posterior anlages with obliteration of the diverticulum from the third ventricle is effected in the fifth prenatal month. As a result of the fusion of the two anlages the pineal body assumes the conical form which characterizes the fully developed stage.

During the sixth month of fetal life there is marked increase in the bulk of the organ. At about the middle of the sixth fetal month large masses of small, deeply staining cells appear throughout the developing organ. With further development these small cells show a characteristic arrangement into cordlike masses, which tend to surround collections of large, less deeply staining cells. This arrangement, the collections of large clear cells (parenchymal cells) surrounded by dense masses of small, deeply staining cells, gives the tissue an appearance strikingly unusual, which has been likened to a mosaic pattern (fig. 3 *A* and *C*).

The further development of the pineal body until birth consists of an increase of the vascularity of the stroma, which is most abundant in the areas containing the small cells, and a moderate increase in the size of the organ, with the collections of large cells becoming relatively more prominent. During the first postnatal month there begins progressive diminution in the number of the small dark-staining cells. By the end of the ninth postnatal month virtually all the small cells have disappeared and there is a marked increase in fibrous tissue in the areas previously occupied by these cells. There is little change in the histologic structure of the pineal body with advancing years. The islands of parenchymal cells remain, the septums of connective tissue surrounding the large cells increase slightly, and focal areas of calcification begin to appear throughout the organ about the second year of life. For a more detailed and complete description of the histogenesis and

7. Krabbe, K. H.: Anat. Hefte **54**:191, 1916.

development of the pineal body the reader is referred to the splendid studies of Krabbe,[7] Marburg[8] and Globus and Silbert.[4]

The large cell is frequently referred to as the "parenchymal cell" of the pineal body (del Río Hortega[9]; Globus and Silbert[4]; Horrax and Bailey[3]), and it is universally agreed that it develops from the pineal anlage and is of nervous origin. Hortega[9] demonstrated in histologic studies that this cell has an unusual type of process with a bulbous ending. Russell and Gregory[10] concluded from comparative histologic studies that it should be regarded as an altered visual cell from the retina of the old pineal eye. Their conclusion is based on the finding of similarly shaped processes on the visual cells from paired eyes of certain vertebrate forms in which the paired eyes have undergone retrogressive evolutionary changes.

The histogenesis of the small cell is disputed. Horrax and Bailey[3] regarded the small cell as a glial cell of neuroectodermal origin which developed with the parenchymal cell in the pineal anlage. Globus and Silbert,[4] on the other hand, after a careful study of the histogenesis of the human pineal body concluded that the small cells were derived from mesenchyme and were capable of differentiating into fibroblasts. These investigators observed that the marginal grouping of the small cells around the large collections of parenchymal cells making up the basic mosaic pattern of the pineal body in the infantile stage disappeared in the maturing organ, the small cells being apparently replaced by fibrous tissue. The disappearance of the small cells was interpreted by them as representing a transformation of the small cells into fibroblasts.

The hypothesis of Globus and Silbert[4] of a mesenchymal origin for the small cells is open to question, since the presence of large collections of mesenchymal cells in the pineal anlage was unexplained by these authors and no migration of mesenchymal cells into the pineal anlage was described in their study of the histogenesis of this structure. Moreover, the transformation of the small cells into fibroblasts in the maturing organ as suggested by them is certainly not the most logical explanation, for with the degeneration and disappearance of the small cells in the adult stage an expected sequence of events would probably be an increase in fibrous tissue. The concept of Horrax and Bailey[3] of a neuroectodermal origin for the small supporting cells that develop from the pineal anlage is more reasonable.

Because the tumors classified as pinealoma characteristically contain the two types of cells observed in pineal tissue at the time of birth, it is concluded that their cells are likewise of neuroectodermal origin. Pinealoma, therefore, is merely another type of glioma representing neoplastic pineal tissue at its highest stage of development, namely, at about the time of birth.

CRITERIA FOR THE DIAGNOSIS OF PINEALOMA

Considerable difference of opinion prevails concerning just which type of primary pineal tumor should be called pinealoma. Horrax and Bailey[3] described a type of pinealoma which was named "spongioblastic pinealoma." They regarded spongioblastic pinealoma as a type of poorly differentiated glial tumor of the pineal anlage occurring before the two types of cells were differentiated. It was admitted, however, that in many instances it was impossible to differentiate spongioblastic pinealoma from glial tumors primary in other parts of the brain. Globus and Silbert,[4] on the other hand, did not recognize even the possibility of such a type of glial tumor arising in the pineal body because they were unable to demonstrate

8. Marburg, O.: Arb. a. d. neurol. Inst. a. d. Wien. Univ. **17**:217, 1908.
9. del Río Hortega, P.: Arch. de neurobiol. **3**:359, 1922.
10. Russell, W. O., and Gregory, W. K.: Unpublished data.

any glial cells in the pineal body in their study of the histogenesis of that organ. In the 7 cases of pineal tumor reported by them the diagnosis of pinealoma was given in every instance. As for those pineal tumors not showing two types of cells or a mosaic pattern, they interpreted those as representing some phase in the differentiation of the cells in the pineal anlage before that showing the two characteristic cells.

Still a third type of supposedly characteristic primary pineal tumor was reported by Baggenstoss and Love.[6] In reporting 10 cases of pineal tumor these authors recognized pinealoma with its two types of cells, the spongioblastic pinealoma of Horrax and Bailey [3] and, in addition, a third type, called by them pineal ependymoma. As can be judged from the description and photomicrographs, this type possessed nothing which would distinguish it from other tumors termed ependymoma.

The most satisfactory classification for any tumor is one based on the cell from which the tumor is believed to originate, as advocated by Mallory [11] and by Ewing.[12] A nomenclature depending on purely descriptive, morphologic criteria or on what the tumor may produce, exemplified by such terms as "round cell sarcoma" and "cholesteatoma," is often misleading and in the opinion of many oncologists not strictly scientific. For this reason we have adopted a rigid concept of what type of tumor is deserving of the name "pinealoma." The designation of a tumor as spongioblastic pinealoma or pineal ependymoma appears to us not justified if there is no definitely distinguishing characteristic feature of the tumor to differentiate it from a glial tumor arising in some other area of the brain. Just because a tumor is situated in the pineal region does not warrant calling it pinealoma. This error occurs frequently in the literature. To illustrate this point, we have recently observed a primary tumor of the pineal body that histologically was typical spongioblastoma multiforme. According to the classification of Horrax and Bailey,[3] the diagnosis for this tumor would be spongioblastic pinealoma. We feel, however, that this tumor is more correctly called spongioblastoma multiforme of the pineal body, for its histologic characteristics were no different from those of spongioblastoma multiforme arising in another part of the brain.

We have been unable to confirm the conclusions of Globus and Silbert [4] that primary tumors of the pineal body not showing two types of cells represent differential stages in the developing pineal body before the appearance of the two types of cells. The possibility is freely admitted, however, that a tumor may arise from pineal tissue which is not sufficiently differentiated to show the two characteristic types of cells. Because the pineal anlage is derived from nerve tissue, such a tumor should exhibit a type of growth resembling some type of glioma. We have observed 4 cases of primary tumor of the pineal body that did not fulfil our criteria of two types of cells, and in each instance it was possible to classify the tumor as some type of glioma. For example, in case 10, reported by Globus [13] as a case of pinealoma, the histologic description and the photomicrograph suggest astrocytoma, and the author described "well differentiated astrocytes."

Since pineal tumors showing two types of cells and a mosaic pattern reproduce pineal tissue in its most highly developed form, we believe that the term "pinealoma" is correctly applied only if it is used exclusively for those pineal tumors containing the two characteristic types of cells seen in normal pineal tissue at the time of birth. The presence of a mosaic pattern is not regarded as necessary for

11. Mallory, F. B.: J. M. Research **13**:113, 1905.
12. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, pp. 9-13.
13. Globus, J. H.: Arch. Path. **31**:533, 1941.

the diagnosis, because occasionally pineal tumors showing the two types of pineal cells will show no mosaic arrangement. The term "pinealoma" as used in this study is based on this concept of the tumor.

LITERATURE AND PREVIOUSLY REPORTED CASES OF PINEALOMA

A review of the literature of pineal tumors reveals 51 cases that in our opinion are verified instances of pinealoma. Our criterion that this type of tumor must have two types of cells was rigidly followed, and in all the cases accepted a photomicrograph or a histologic description verified the presence of the two characteristic cells. No doubt, several more cases of pinealoma have been reported that are not included here, for in many instances the histologic description of the tumor was inadequate to establish the diagnosis by our criteria, and in other instances a histologic description was not given. Baggenstoss and Love [6] reported 10 cases of pineal tumor but gave a histologic description of the tumor in only 4. Horrax [14] reported a case in which he gave pinealoma as the pathologic diagnosis but did not give a histologic description. We were unable to obtain the original publications of Hempel [15] and Steiner and Johan.[16] The collected cases, including the 7 reported in this paper, are listed in table 2.

STUDY OF THE COLLECTED CASES

The general features of pinealoma can be determined by an analysis of this group of 58 cases (tables 1 and 2) under the following heads:

Age and Sex.—As judged from the cases collected for this study, pinealoma occurs most frequently in young adults between the ages of 15 and 25 years (28 cases). It is seen much less frequently in persons at or under 15 years (17 cases) and beyond the age of 25 years (13 cases). It occurs preponderantly in males (88 per cent); only 5 of the 58 tumors collected occurred in females.

Symptoms.—The general signs of increased intracranial pressure were present in nearly all instances. Headache was the first symptom noted by 41 of the 58 patients and was associated with vomiting in 18. Other symptoms, such as polyuria or a symptom referable to one or more cranial nerves without headache and vomiting, were the first symptoms complained of by only 13 patients. Paralysis of one or more cranial nerves was observed in 36 patients. Disturbance of vision or papilledema was the most constantly observed neurologic sign, for such disturbances were statedly absent in only 6 of the cases. Most interesting is the observation that precocious puberty was present in only 3 of the 17 patients at or below the age of 15 years. Diabetes insipidus was observed in 15 of the patients, and other symptoms indicating some endocrine imbalance, such as obesity or hypogonadism, were noted in 10. Loss of the ability to look upward was specifically mentioned in 21 cases, not mentioned in 29 and definitely stated to be not present in only 8 instances. This symptom is indicative of a lesion in the posterior part of the third ventricle and frequently is noted with pineal tumors.

Rate of Growth.—As can be judged from the duration of the patients' symptoms before seeking medical aid, the tumors classified as pinealoma are fairly rapidly growing ones, for 32 patients had symptoms that could be attributed to intracranial disease for less than a year, and 20 of these, for less than six months. In 22 patients the symptoms were noted for over a year, the longest period being

14. Horrax, G.: Arch. Neurol. & Psychiat. **37**:385, 1937.

15. Hempel, H. K.: Ein Beitrag zur Pathologie der Glandula Pinealis, Inaug. Dissert., Leipzig, B. Georgi, 1901.

16. Steiner and Johan: Orvosi hetil. **66**:367, 1922.

TABLE 2.—*Reported Cases of Pinealoma*

Number	Author	Age of Patient	Sex	Initial Symptoms: Headache	Initial Symptoms: Vomiting	Initial Symptoms: Others	Important Neurologic Signs: Cranial Nerve Paralysis	Important Neurologic Signs: Loss of Upward Gaze	Important Neurologic Signs: Visual Disturbances and Papilledema	Endocrine Disturbances: Precocious Puberty	Endocrine Disturbances: Diabetes Insipidus	Endocrine Disturbances: Obesity or Glandular Disturbances	Duration of Symptoms before Treatment	Treatment: Operation	Treatment: Irradiation	Location: Pineal Region 3d Ventricle	Location: Lateral Ventricle	Location: Subarachnoid Space	Autopsy	Mosaic Pattern	Final Result
1	Reinhold, H.: Deutsches Arch. f. klin. med. **39**: 1, 1886..	19	M	+	0	+	+	+	+	0	0	0	7 mo.	0	0	+	0	0	+	0	Death
2	Howell, C. M. H.: Proc. Roy. Soc. Med. **3**: 65, 1910 Case 1	42	M	+	0	+	+	+	+	0	0	0	6 mo.	+	0	+	0	0	+	?	Death
3	Case 2	22	M	0	+	+	+	+	+	0	+	0	3 mo.	?	0	+	0	0	+	?	Death
4	Case 3	20	M	+	0	0	?	+	+	0	0	0	5 mo.	+	0	+	+	0	+	?	Death
5	Rorschach, H.: Beitr. z. klin. Chir. **83**: 451, 1913	27	M	0	0	+	+	?	+	0	0	0	2 yr.	+	0	+	+	0	+	+	Death
6	Uemura, S.: Frankfurt. Ztschr. f. Path. **20**: 380, 1917..	59	M	?	?	?	?	?	?	0	?	?	?	?	?	+	+	0	+	?	Death
7	Skoog, A. L.: New York M. J. **107**: 1199, 1918	9	M	+	+	0	0	?	+	+	0	+	4 mo.	0	0	+	0	0	+	?	Death
8	Löwenthal, K.: Beitr. z. path. Anat. u. z. allg. Path. **67**: 207, 1920	23	M	+	0	0	+	?	+	0	+	+	4 mo.	0	0	+	0	0	+	+	Death
9	Berblinger, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **95**: 741, 1925	33	M	+	0	+	+	?	+	0	0	0	1 yr.	0	0	+	0	+	+	+	Death
10	Horrax and Bailey [8] (1925) Case 4	12	M	+	+	0	+	?	+	+	+	0	3 mo.	0	0	+	0	0	+	+	Death
11	Case 7	23	M	+	0	+	+	?	+	0	0	0	1 yr.	+	0	+	0	0	+	+	Death
12	Case 8	13	M	+	+	+	?	+	+	0	+	0	3 yr.	+	0	+	0	0	+	++	Death
13	Case 9	17	M	+	+	0	+	?	+	0	+	0	1 yr.	0	0	+	0	0	+	+	Death
14	Case 10	20	M	+	+	0	+	+	+	0	+	0	9 mo.	+	0	+	0	0	+	+	Death
15	Balado, M.: Arch. argent. de neurol. **1**: 10, 1927 Case 1	17	M	+	0	0	+	+	+	0	0	0	3 mo.	+	0	+	0	0	+	++	Death
16	Case 2	15	M	0	0	+	+	+	+	0	+	0	1 yr.	0	0	+	+	0	+	+	Death
17	Arend, R., and Schusterówna, H.: Polska gaz. lek. **9**: 381, 1930	10	M	+	0	0	+	?	+	0	0	+	2 yr.	0	0	+	0	0	+	?	Death
18	Fulton, J. F., and Bailey, P.: J. Nerv. & Ment. Dis. **69**: 1, 1929 (case 2)	28	F	0	0	+	0	0	+	0	+	+	5 yr.	+	0	?	+	0	+	?	Death
19	Liebert: Deutsche Ztschr. f. Nervenh. **108**: 101, 1929....	10	M	+	+	0	+	+	0	0	0	0	6 mo.	0	0	+	0	0	+	+	Death
20	Kux, E.: Beitr. z. path. Anat. u. z. allg. Path. **87**: 59, 1931	22	M	+	+	+	+	?	+	0	0	0	6 mo.	0	0	+	0	0	+	0	Death
21	Harris and Cairns (1932)[19]	20	M	+	0	0	?	0	+	0	0	0	2 yr.	+	+	+	?	?	0	++	Indeterminate, well after 2 yr.
22	Globus (1932)[17]	2	M	0	0	+	+	?	+	0	0	0	2 mo.	+	0	+	+	0	+	0	Death
23	Guillain, G.: Vol. jubilaire, Marinesco, 1933, p. 281	25	M	+	+	0	+	+	+	0	0	0	2 yr.	0	0	+	0	0	+	0	Death
24	Vincent, C., and Rappoport: Rev. neurol. **1**: 517, 1933...	40	M	0	0	+	+	?	+	0	0	0	2 yr.	+	0	+	0	0	+	?	Death
25	Stringer, S. W.: Yale J. Biol. & Med. **6**: 375, 1934	27	M	0	0	0	0	0	0	0	+	0	6 mo.	0	0	+	0	+	+	+	Death

26	Friedman, E. D., and Plaut, A.: Arch. Neurol. & Psychiat. **33**: 1324, 1935	33	M	0	0	+	0	?	+	0	+	+	4 yr.	0	0	+	0	+	+	0	Death
27	Benedek, L.: Ztschr. f. d. ges. Neurol. u. Psychiat. **156**: 677, 1936	26	?	0	0	+	?	?	+	0	0	+	2 yr.	+	0	+	0	0	+	++	Death
28	Dandy (1936)[5]																				
	Case 1	10	M	+	+	0	+	+	?	0	0	0	1 mo.	+	0	+	?	?	0	+	Death
29	Case 2	28	F	+	0	0	+	?	+	0	0	0	7 mo.	+	0	+	?	?	0	?	Death
30	Case 3	15	M	+	+	+	+	+	+	0	0	0	6 yr.	+	0	+	?	?	0	+	Indeterminate
31	Groff, R. A.: Am. J. Cancer **29**: 651, 1937	18	M	0	0	+	+	+	+	0	+	+	2 yr.	0	0	+	+	0	+	+	Death
32	van Gehuchten, P., and Callervaert, P.: J. belge de neurol. et de psychiat. **37**: 283, 1937																				
	Case 1	9	M	+	0	0	+	?	+	0	0	0	2 yr.	+	0	+	0	0	+	++	Death
33	Case 3	19	M	+	0	0	+	+	0	0	0	0	2 yr.	+	0	+	0	0	+	?	Death
34	Alajouanine, T.; Hornet, T., and Thurel, R.: Rev. neurol. **68**: 793, 1937	29	M	+	+	0	+	?	0	0	0	0	2 mo.	0	0	+	+	+	+	+	Death
35	Urechia, M. C. I.: Rev. neurol. **70**: 511, 1938	50	M	+	0	0	+	?	+	0	0	0	?	0	0	+	0	0	+	+	Death
36	Cleghorn, R. A.: Hyland, H. H.; Mills, J. R. F., and Linell, E. A.: Quart. J. Med. **7**: 183, 1938	23	M	+	0	+	0	0	0	0	0	+	1 yr.	0	0	+	0	0	+	+	Death
37	Baggenstoss and Love (1939)[6]																				
	Case 2	22	M	0	0	+	+	?	+	0	+	0	9 mo.	0	0	+	+	+	+	++	Death
38	Case 3	18	M	+	0	0	+	?	+	0	0	+	10 mo.	+	+	+	+	+	+	+	Death
39	Cabot case 25302, New England J. Med. **221**: 151, 1939	29	M	+	0	+	0	+	+	0	0	0	3 mo.	+	0	+	0	0	+	?	Death
40	Mackay, R. P.: Arch. Neurol. & Psychiat. **42**: 842, 1939.	18	M	0	0	+	0	0	0	0	0	0	3 yr.	0	0	+	+	0	+	0	Death
41	Akamatu, H.: Gann **33**: 371, 1939	23	M	0	0	+	?	?	?	0	+	+	10 mo.	0	0	+	+	0	+	++	Death
42	Prado, J. M.: Arch. argent. de neurol. **22**: 21, 1940																				
	Case 1	17	M	+	0	+	0	0	+	0	0	0	3 mo.	+	0	0	+	0	+	0	Death
43	Case 4	18	M	+	0	+	+	?	+	0	0	0	6 mo.	+	0	+	0	0	+	0	Death
44	Sato, H.: Gann **35**: 351, 1941	18	M	+	+	0	?	?	?	0	0	0	1 mo.	0	0	+	+	0	+	+	Death
45	Globus (1941)[13]																				
	Case 1	13	M	+	0	0	0	?	+	0	0	0	2 yr.	+	0	+	+	0	+	++	Death
46	Case 2	17	M	+	+	+	+	+	+	0	+	0	4 mo.	+	0	+	+	0	+	++	Death
47	Case 3	13	M	+	0	0	+	+	+	+	0	0	2 yr.	+	0	+	0	0	+	0	Death
48	Case 4	16	M	+	0	0	?	?	?	0	0	0	?	+	0	+	0	0	+	+	Death
49	Case 5	30	M	+	0	0	+	?	+	0	0	0	2 mo.	+	0	+	0	0	+	0	Death
50	Case 6	2	M	?	+	+	?	?	+	0	0	0	1 mo.	0	0	+	0	0	+	0	Death
51	Case 12	16	F	+	+	0	+	+	+	0	0	0	1 yr.	0	+	+	+	0	+	0	Death
52	Russell, W. O., and Sachs, E.: Arch. Path. **35**: 869, 1943																				
	Case 1	24	M	+	+	+	+	+	+	0	0	0	6 mo.	+	0	+	0	0	+	0	Death
53	Case 2	17	M	+	0	0	?	?	?	0	0	0	?	0	0	+	+	0	+	+	Death
54	Case 3	17	M	+	+	0	+	+	+	0	0	0	3 mo.	+	0	+	0	0	+	+	Death
55	Case 4	14	M	+	+	0	?	+	+	0	0	0	5 mo.	+	0	+	0	0	+	++	Death
56	Case 5	10	F	0	0	+	+	?	+	0	+	0	5 mo.	+	+	+	?	+	?	+	Death
57	Case 6	9	M	+	+	0	0	0	+	0	0	0	4 mo.	+	0	+	0	0	+	++	Death
58	Case 7	2	F	0	0	+	0	0	+	0	0	0	2 mo.	+	0	+	?	?	0	+++	Indeterminate

six years (Dandy,[8] case 3). Apparently there is no correlation between the duration of the disease before the patient comes to the hospital and the type of growth and differentiation of the tumor, for in case 7 (table 1) of our series the total duration of the symptoms was only two months and the tumor showed the most highly developed and differentiated type of growth.

Gross Appearance and Growth Behavior.—The gross appearance of the tumor of this group of 58 cases was extremely variable. In some instances it was a small encapsulated mass of tissue, not much larger than the pineal body itself, which remained localized and caused symptoms only by pressure on adjacent structures (fig. 1 *A*). In other instances it was disseminated throughout the lateral ventricles (18 cases) (fig. 1 *B*) and into the cerebral and spinal subarachnoidal spaces (7 cases). The usual finding, however, was a nonencapsulated infiltrating tumor in the region of the pineal body on the habenular commissure, which had extended either anteriorly into the third ventricle or posteriorly to involve the corpora quadrigemina and the interbrain structures.

Microscopic Appearance.—The discussion of the histologic features of pinealoma and its cellular types given in this section is based on the 7 cases of pinealoma observed by us. Only in regard to the mosaic pattern was it possible to include the results of a study of the cases collected from the literature. The histologic structure of pinealoma is extremely variable since it consists of cells of two distinctly different types each of which may show individual pleomorphism along with a quantitative difference in the ratio of the cells of one type to the cells of the other. Still another variable was the tendency of the cells in most instances to show a characteristic morphologic arrangement that has been likened to a mosaic pattern. The variations observed in each cell and the mosaic pattern are discussed separately.

The large cells (parenchymal cells) showed great variation in outline and form in different tumors and oftentimes in the same tumor, so that no one description can be given that will adequately cover the wide morphologic variation. The large cells in cases 4, 5 and 6 (table 1) were round to oval, with clear cytoplasm and a round, moderately chromatic nucleus (fig. 2 *A*). Because of the clear halo of cytoplasm, this type of cell strikingly resembled the type cell of oligodendroglioma. It showed no intracellular or extracellular fibrils when stained with phosphotungstic acid–hematoxylin; however, it frequently contained small round or slightly elongated blepharoplastic granules (fig. 2 *D*). In other tumors (cases 1, 2 and 3 of table 1) the large cells were associated usually with a moderate number of deep blue–staining intercellular fibrils when stained with phosphotungstic acid–hematoxylin (fig. 2 *C*). Mitotic figures were present in moderate numbers in the large cells in 6 of the tumors, but mitotic figures were not seen in the tumor in case 7. This fact is highly significant, for this tumor was the most highly differentiated tumor of the group and more closely resembled pineal tissue as seen at the time of birth than any of the other tumors studied. Here the large cells showed still another type of variation and more closely resembled the parenchymal cells in the pineal tissue present at birth than did cells in any of the other tumors. This type of large cell contained a small round hyperchromatic nucleus with clear cytoplasm having poorly defined borders (fig. 3 *B*).

Because the large cells in pineal tissue have characteristic processes with bulbous endings which are demonstrated only with the silver impregnating technics, attempts were made to show similar processes on the large cells in pinealoma. In none of the 7 tumors studied, however, were we able to demonstrate any processes resembling those described by Hortega[9] for pineal cells. Processes resembling

those of normal pineal cells have been described on the large cells of pinealoma by Horrax and Bailey.[8] Bielschowsky's staining method for neurofibrils, Hortega's method for pineal parenchyma and Hortega's method for astrocytes were tried on each tumor without results. Characteristic processes were shown, however, on the large pineal cells in normal pineal tissue by these methods.

The small cells in most instances were indistinguishable from small or large lymphocytes. They were characteristically round and contained moderately basophilic cytoplasm with a hyperchromatic round or slightly indented nucleus. In most of the tumors the small cells showed gradations in size from a cell resembling a large lymphocyte, on the one hand, to a cell resembling a small lymphocyte, on the other (fig. 3 *B*). Careful study of all the tumors disclosed no mitotic figures in the small cells. Because the small cells in pinealoma so closely resemble lymphocytes the question is reasonably asked whether they are truly neoplastic cells and part of the tumor or actually lymphocytes that have infiltrated the tumor as is occasionally seen in some types of epithelial tumors. We believe that they are neoplastic cells because they resemble the small cells in infantile pineal tissue, which are admittedly not lymphocytes but a type of glial cell. Moreover, they show a characteristic arrangement in the tumor that reproduces the mosaic pattern of infantile pineal tissue, which further identifies them with pineal tissue.

The mosaic pattern, shown in 34 of the 58 tumors, in its most highly differentiated form was a complete reproduction of the cellular arrangement in pineal tissue as observed at the time of birth (fig. 2). In only 13 of the 58 tumors was there no discernible mosaic pattern. The description was not complete enough in 11 cases to allow one to be certain whether a mosaic arrangement was present or not. Those tumors showing a poorly developed mosaic pattern were regarded as examples of a more anaplastic type of tumor. The age of the patient apparently was not a determining factor in the development of the mosaic pattern, for the tumors from some of the youngest (Globus [17]) and oldest patients (Friedman and Plaut [18]) showed no mosaic pattern. The mosaic pattern can be regarded as a fairly constant feature of pinealoma since 34 of the 58 tumors showed some semblance of it. The absence of a mosaic pattern does not in any way vitiate the diagnosis of pinealoma if the tumor contains the two characteristic cells. In fact, the point should be strongly emphasized that in some instances, as was observed in cases 4 and 5 (table 1), large areas of the tumor may be composed solely of the large cells without any small cells, while in other areas of the tumor the two types of cells will form a fairly characteristic mosaic pattern.

Treatment and Prognosis.—Thirty-two of the 58 patients were treated by operation. High voltage roentgen radiation was used in the treatment of 4 patients, but no improvement that could be attributed to the rays was noted. Death was known to be the final result in all of the cases except 3 (cases 21, 30, 58, table 2). In case 21 (Harris and Cairns [19]) the patient completely recovered from the operation for removal of the tumor and was reported well, without further detail, two years after the operation. In case 30 (Dandy [5]) the patient recovered from the operation, but details of the patient's subsequent course are not reported. It is not known whether a permanent cure was effected in case 58, contributed by us, since the patient could not be located after she left the hospital, although she was symptom free and recovered from the operation at the time of discharge.

17. Globus, J. H., in Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman, New York, International Press, 1932, vol. 2, p. 491.
18. Friedman, E. D., and Plaut, A.: Arch. Neurol. & Psychiat. **33**:1324, 1935.
19. Harris, W., and Cairns, H.: Lancet **1**:3, 1942.

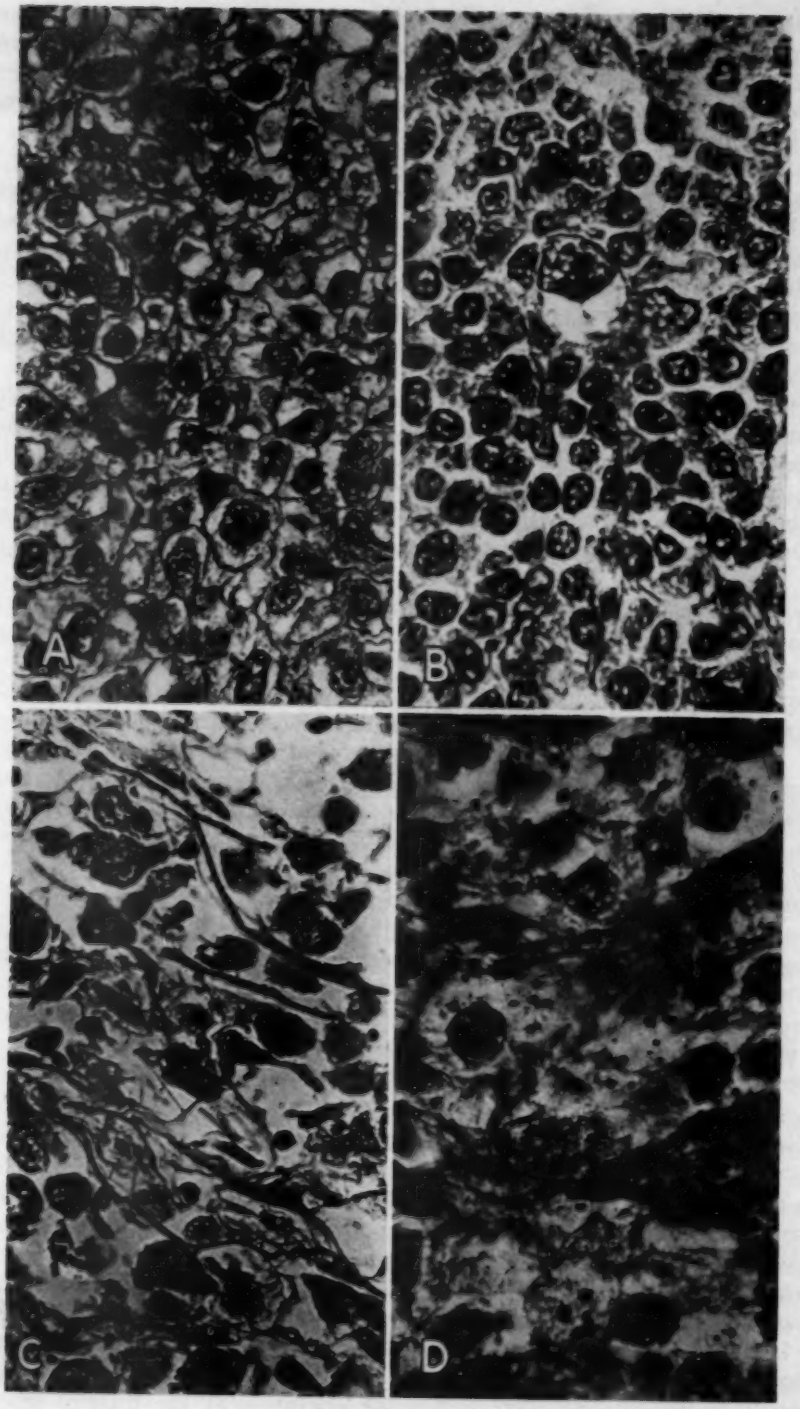

Figure 3

(See legend on opposite page)

Since death was known to be the final outcome in all but 3 cases, it is clear that at present pinealoma offers an extremely poor prognosis. The location of the growth in the roof of the third ventricle is an important factor in accounting for its extremely poor prognosis, because extension of the tumor to the third ventricle or the interbrain structures is the rule, so that complete surgical removal is impossible.

We have had personal communications with German, Dandy and Horrax concerning their experiences with pinealoma. German cited a patient whom he treated by operation who is living and free from evidence of recurrence five years after the removal of the tumor. Dandy mentioned a patient who is living and well five and a half years after operation and others who have lived for two to three years following operation. Horrax reported a young woman who is well and without symptoms of disease five years after the removal of a tumor diagnosed as pinealoma and a second patient who lived for eight years after removal of a tumor with this diagnosis. In the latter's case roentgen treatment was given immediately after operation, for it was known that all the tumor was not removed. The patient improved with the roentgen therapy and was well for five years when symptoms of intracranial disease returned. She survived three more years and died following an operation for radical removal of her tumor eight years after the first operation.

COMMENT

In this study three points merit special comment and consideration: first, the part played by pinealoma in the production of the precocious puberty associated with pineal tumors; second, a discussion of the prevailing concept of the physiologic mechanisms involved in this syndrome, and third, the unusual type of neoplasia shown by pinealoma.

With the collection of 58 histologically verified cases of pinealoma opportunity is afforded to determine whether or not the tumor itself is directly concerned with the syndrome of precocious puberty. The implied reasoning often encountered is that the pineal body is some form of endocrine gland and neoplasia of its tissue produces a tumor which in turn elaborates a hormone that is directly responsible for the precocious sexual development. If such a hypothesis is correct, only pinealoma, representing neoplasia of pineal tissue, should be significant, since other types of tumor arising in the pineal body, such as glioma or teratoma, are known not to be associated with precocious puberty when observed in other parts of the brain (glioma) or the body (teratoma).

EXPLANATION OF FIGURE 2

In this figure a comparison is made between pineal tissue at the time of birth and pinealoma.

A, section of pineal tissue from a full term infant. Collections of the large pineal cells are surrounded by cordlike collections of the small cells. The arrangement of small and large cells gives the characteristic mosaic pattern. Hematoxylin and eosin stain; × 160.

B (case 7), section of the tumor showing large and small cells forming a mosaic pattern. Note that the blood vessels visible in the section are all contained in the areas of the small cells. Compare with *A*, showing pineal tissue at the time of birth. Hematoxylin and eosin stain; × 160.

C, higher magnification of a part of the section shown in *A*. The large cells are shown in the upper and lower parts of the field with a mass of small cells, deeply basophilic in staining, in the center of the section. Hematoxylin and eosin stain; × 570.

D (case 5), section showing large cells in the lower right side of the field and small cells in the upper left side. Note the mitotic figure in the center of the group of the large cells in the lower right center position and the characteristic grouping of the large and the small cells. Compare with *C*. Phosphotungstic and hematoxylin stain; × 510.

The first point to be considered is the fact that pinealoma is not a tumor of preadolescent years but has its highest incidence (48 per cent of all cases) in the first ten year period following puberty. There is no demonstrable histologic change in the pineal body occurring after puberty that could account in any way for the high incidence of pineal tumors in this period. Only 17 of the 58 patients (29 per cent of all cases) were at or below the age of puberty; so from a purely statistical standpoint there has been but a small number of tumors diagnosed as pinealoma that could have produced precocious puberty. Certainly the most remarkable finding in this study is that of the 17 patients with pinealoma at or below the age of 15 years only 3 showed associated precocious sexual development.

The observations reported by Bing, Globus and Simon [2] on teratoma of the pineal body are cited for comparison with those on pinealoma. In a complete review of all the reported cases of pineal tumor in the literature in 1938 these authors found that there were 18 cases of teratoma of the pineal body in which the patient was at or below the age of 15 years and in 10 of these precocious physical and sexual development was present. This would indicate that teratoma, which is not known to be associated with precocious puberty when occurring in other parts of the body, is responsible for precocious puberty more frequently than is pinealoma. This observation is of major importance in considering the question of pineal tumors and disturbed endocrine functions since it indicates plainly that pinealoma produces precocious puberty less frequently than another type of tumor primary in the pineal body.

If the supposition is correct that the pineal body is an endocrine gland and its tumor a functioning one, it is reasonable to expect endocrine changes not only in preadolescent life but after puberty as well. No endocrine changes have been observed in the older patients suggesting hormonal disturbances. The only disturbed endocrine function noted in the adult patients was diabetes insipidus, and this disease was noted in the same incidence in the patients below puberty. Moreover, diabetes insipidus is known to be a syndrome associated with hypothalamic lesions and is frequently seen in patients who have had long-standing hydrocephalus with resulting pressure on the hypothalamus.

From the foregoing comment it is plainly seen that pinealoma is not a functioning tumor and is associated with precocious puberty less frequently than are other types of primary tumor of the pineal body. Yet precocious puberty has been reported more frequently with pineal tumors and even with pinealoma than with tumors occurring in other parts of the brain. This fact is strongly attested by the study of Bing, Globus and Simon,[2] who collected 177 cases of pineal tumor from the literature. In their study 21 of the 41 patients at or below the age of 15 years showed the syndrome of precocious sexual development. Primary pineal tumors are not the only intracranial tumors associated with precocious puberty, for tumors and non-neoplastic lesions involving the hypothalamus are occasionally associated with precocious sexual development even though the pineal body is unaffected. Weinberger and Grant [20] reported such a case and were able to collect 16 other cases from the literature. From their study of hypothalamic lesions associated with precocious puberty and a general consideration of the problem, including pineal tumors, they advanced a reasonably sound hypothesis to explain this remarkable association of precocious sexual development with intracranial tumors. According to Weinberger and Grant,

> . . . Lesions of the posterior portion of the hypothalamus interrupt nerve pathways or interfere with mechanisms which normally inhibit and control the production and release of

20. Weinberger, L. M., and Grant, F. C.: Arch. Int. Med. **67**:762, 1941.

gonadotropic substances from the anterior lobe of the hypophysis. The excessive liberation of these pituitary substances stimulates the ovaries or the interstitial cells of the testes, as the case may be, to overproduction of their specific principles, the estrogenic and androgenic substances. The latter are the substances known to be responsible for the development of the secondary sexual characters.

This explanation is without experimental proof on several points, yet it does offer a satisfactory working hypothesis. Furthermore, it clearly localizes the mechanism for precocious sexual development in the hypothalamus and not in the pineal body. The work of Bing, Globus and Simon [2] offers further support for the conclusion reached by Weinberger and Grant.[20] In 21 cases of precocious sexual development associated with pineal tumor collected from the literature they found that the tumor invariably involved some adjacent structure, such as the corpora quadrigemina, the thalamus or the floor of the third ventricle. No one of these structures was more frequently involved than the others, so that no definite statement concerning the exact location of the mechanism could be made from their study. For a complete review of the reported pathologic data on hypothalamic lesions and precocious puberty and on the role played by the hypophysis, the reader is referred to the paper of Weinberger and Grant.[20]

From a purely pathologic standpoint pinealoma is a unique and interesting type of tumor because it is an example of an autonomous new growth of a whole tissue. That is to say, it contains the same two characteristic cells found in pineal tissue, which in many instances tend to arrange themselves into a characteristic form resembling the mosaic pattern seen in pineal tissue at the time of birth. This unusual characteristic of pinealoma was best shown in case 7 (table 1), in which the new growth was the most striking example of complete reduplication of normal pineal tissue of all the tumors we have studied. Generally, a tumor with cells showing unmistakable autonomy of growth is composed of neoplastic cells of a single type. The individual cells may show extreme pleomorphism with special and varied types of differentiation as in osteogenic sarcoma, in which chondroblasts, osteoblasts and fibroblasts may be intermingled in the same tumor. To illustrate this point further, if carcinoma of the stomach were to reduplicate gastric mucosa to the same degree as pinealoma reduplicates pineal tissue, that tumor would contain glandular structures composed of well differentiated chief and parietal cells that show a tendency to arrange themselves in parallel rows resembling normal gastric mucosa.

Globus and Silbert [4] have referred to this unusual quality of pinealoma as indicating an autochthonous teratoid type of tumor. The term "teratoid" connotes a type of tumor that may reproduce a whole tissue yet cannot correctly be regarded as teratoma because there are not present cells representing all three germ cell layers, hence the term "teratoid." The word "autochthonous" refers to the fact that the tumor is producing exactly the type of tissue from which it originates.

There are only two other types of tumor to our knowledge that show true neoplasia of two different types of cells and represent autonomous new growth of a whole tissue. They are chorioma, with its syncytial and Langhans types of cells, and thymoma, containing thymocytes and collections of epithelial cells resembling Hassall's corpuscles. Neither one of these, however, reproduces the normal tissue as completely as does pinealoma. Chorioma, for example, does not show arrangement of its cells to form functioning blood sinuses as this is seen in normal placenta. Neither does thymoma exactly reproduce normal thymic tissue, for the epithelial cells are usually scattered throughout the tumor in small groups that do not exactly reproduce Hassall's corpuscles. That the ability of

these tumors to produce two types of cells is a fundamental and inherent quality is shown by the fact that their metastases in distant organs show the same characteristic two types of cells.

The remarkably high incidence of pinealoma in males (53 males and only 5 females) is difficult to interpret or explain. There is no known fact to indicate that androgen could contribute to the development of neoplastic disease in the pineal body although the extremely high incidence of pinealoma in males is itself excellent presumptive evidence. Pinealoma will have to be placed with the other types of tumor that show high incidence in males, such as carcinoma of the lung, carcinoma of the lower lip and carcinoma of the esophagus, to await further investigation.

SUMMARY

The term "pinealoma" should be reserved exclusively for those primary tumors of the pineal body that reproduce pineal tissue containing two types of cells, which frequently show the characteristic arrangement of a mosaic pattern. Seven such tumors are reported here. In all there were the two characteristic types of cells, and in 6, a mosaic pattern was discernible. A detailed histologic study of these tumors is presented.

In a review of the literature, 51 previously reported tumors diagnosed as pinealoma have been collected: To this group we have added the aforementioned 7. The clinical and pathologic features of this series of tumors are analyzed.

The salient features of pinealoma are that it occurs predominantly in males (88 per cent) between the ages of 15 and 25 years (48 per cent of cases) and that it arises in the pineal body and produces its symptoms by obstructing the aqueduct of Sylvius, thus causing internal hydrocephalus. The initial clinical symptoms produced from the blocking of the aqueduct of Sylvius by the tumor are those of increased intracranial pressure—headaches, vomiting and disturbances of vision. Disturbances of vision and loss of the ability to look up are prominent features of the clinical symptom complex and are due to the frequent involvement of the corpora quadrigemina by the tumor. But there is no group of symptoms that may be considered characteristic of pinealoma. The diagnosis must be made with air studies. Precocious puberty was observed in only 3 of the 17 patients at or below the age of 15 years. Diabetes insipidus was a more frequent complication, with 15 of the 58 patients showing this symptom. This study indicates that pinealoma is not a functioning tumor and plays no direct part in the production of precocious puberty. It is concluded that the mechanism for the production of precocious sexual development associated with intracranial tumors is not directly concerned with pineal tumors or with the pineal body itself but is inherent in the midbrain structures (probably the hypothalamus), because all types of tumors primary in the pineal body as well as of tumors in adjacent structures have been at times accompanied by precocious sexual development.

Pinealoma is a unique type of tumor since it is an example of an autonomous new growth of a whole tissue. It may remain localized in the region of the pineal body; it may be disseminated throughout the third and the lateral ventricles, or it may metastasize to the cerebral and spinal subarachnoidal spaces. Because of its location in the roof of the third ventricle and because of its tendency to spread and metastasize through the central nervous system, it offers little opportunity for complete operative removal. The prognosis is poor, and the cases carry a high mortality rate. Death was known to be the final outcome in 55 of the 58 cases collected.

This study offers no support for the concept that the pineal body is some form of endocrine gland.

ACUTE ULCERATION OF THE ESOPHAGUS WITH ASSOCIATED INTRANUCLEAR INCLUSION BODIES

REPORT OF FOUR CASES

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AND
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BROOKLYN

Acute ulceration of the esophagus is not uncommonly encountered at autopsy. The ulcers are usually superficial erosions and only occasionally appear to have been present for any length of time. Often they are at the level of the larynx and surrounding cartilaginous structures, and in the cases with this location especially there is frequently a history of a stomach tube having been used for feeding or for relief of distention. Bacterial stains reveal a variety of organisms, and yeasts and fungi are sometimes observed.

Recently, however, we have encountered 4 cases of acute esophageal ulceration which differ from the common variety in that well formed intranuclear inclusion bodies similar to those associated with the lesions of virus infections could be found in the epithelium bordering on the ulcer. Gram, methylene blue and Giemsa stains failed to reveal bacteria or other visible microscopic organisms. Since the presence of inclusion bodies and the accompanying characteristic nuclear alteration are such good presumptive evidence of the viral origin of these ulcers, it seems worth while to report this unusual occurrence.

REPORT OF CASES

CASE 1.—A 30 year old white man was admitted to the Long Island College Hospital March 22, 1942. Five months before, he had begun to have abdominal cramps and diarrhea. The stools increased in frequency until he was having from ten to fifteen a day. They were watery and contained blood and mucus. On his admission blood-stained fecal fluid was oozing almost constantly from the rectum. Four weeks earlier he had passed a large amount of fresh red blood and had spent two weeks in another hospital, where he received twelve transfusions. The stools contained no evidence of Endamoeba histolytica. The colon bacillus and an unidentified member of the Salmonella group were cultured repeatedly in large numbers. Cultures were negative for typhoid, paratyphoid A or paratyphoid B bacilli and the dysentery group. Widal tests were negative.

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The patient was in an extreme degree of emaciation and cachexia when he entered the hospital, and he died nine days later. The diagnosis was nonspecific chronic ulcerative colitis.

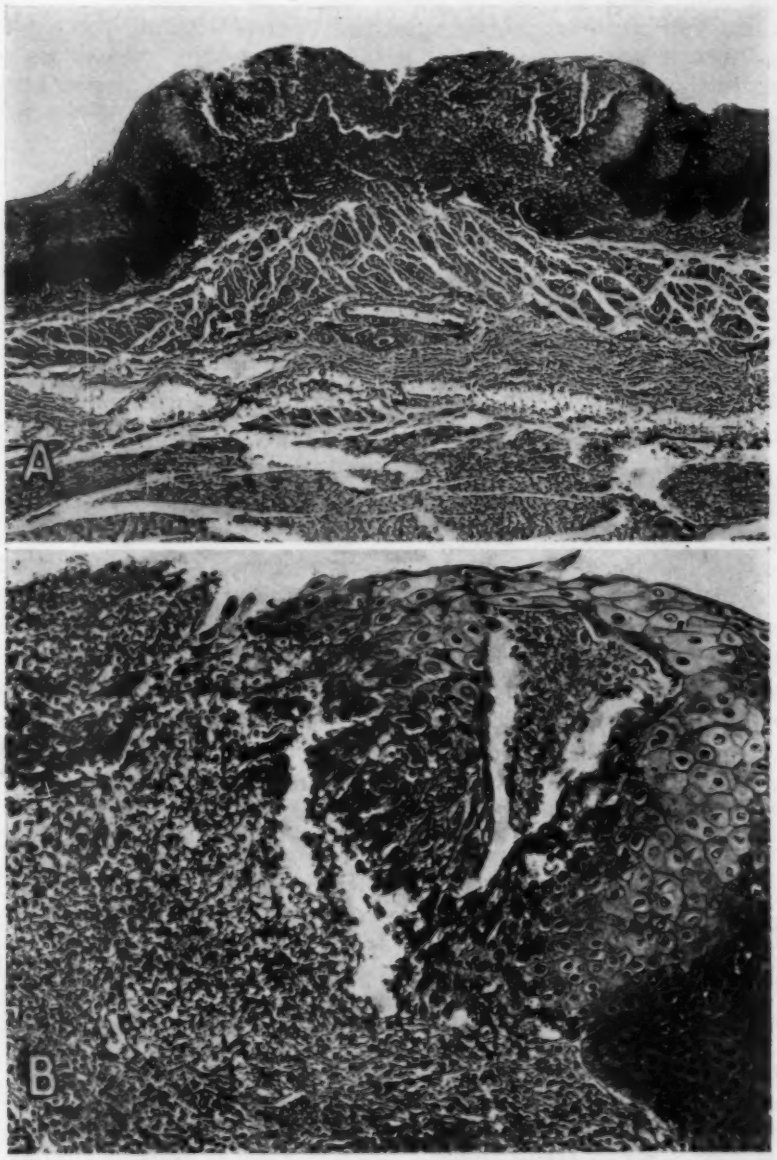

Fig. 1.—*A*, esophageal ulcer in case 1. Hematoxylin and eosin; × 35. *B*, margin of the ulcer in *A*. Intranuclear inclusion bodies can be made out in the zone of pale swollen epithelial cells at the border of the necrotic region, especially in the deeper part of the epithelial layer. Hematoxylin and eosin; × 150.

Autopsy was performed four and one-half hours after death. Extensive ulceration was present throughout the entire colon, beginning at the ileocecal valve and extending to the rectum. It was more pronounced in the cecum, the transverse colon and the descending colon, where only a few shreds of mucous membrane remained. In many areas the ulceration extended through the muscular coat of the intestine. Lying over the bladder and anterior to the rectum was a well localized abscess containing 100 cc. of thick yellowish green purulent material. Culture of this pus and of the colonic ulcers again revealed only the colon bacillus and the same unidentified member of the Salmonella group which had been found in the stools. It was not possible to demonstrate a point of perforation in the intestinal wall which might have given rise to the pelvic abscess.

A moderately extensive lobular or bronchial pneumonia was found in both lungs.

The esophagus was lined by longitudinally wrinkled, pale pink mucous membrane. In the lower 4 cm. of the mucosal surface, especially along the apexes of the wrinkles, there were superficial rounded ulcers, 1 mm. to 4 mm. in diameter, yellow and apparently covered with fibrinous exudate.

Microscopically, the mucosal erosions of the esophagus were found to extend only through the epithelium (fig. 1 *A*). The intervening space was filled with a network of fibrin, in the meshes of which lay necrotic cell fragments, large mononuclear cells, lymphocytes and rare polymorphonuclear leukocytes. At the base of the ulcer there was a small amount of granulation tissue with a peripherally decreasing lymphocytic infiltration. The squamous epithelial cells at the margins of the ulcer stained more palely than those in the unaffected mucosa (fig. 1 *A* and *B*), and in the nuclei of many of them there were inclusion bodies (fig. 2). These bodies took a dark purplish red stain with hematoxylin and eosin. They were round or oval, conforming in shape to that of the nucleus in which they were contained. At the periphery of the centrally located body there was a clear zone which took no stain. The nuclear membrane was dark and distinct, and the remaining chromatin was concentrated immediately inside of it, sometimes giving its inner circumference a beaded appearance. This arrangement formed the "halo" which characteristically surrounds viral intranuclear inclusions. In other cells the inclusion filled the entire nucleus, making it a homogeneous purplish red body, around which was the dark and frequently beaded nuclear membrane.

No bacteria could be found in the esophageal ulcers with Gram, Giemsa or methylene blue stains except in the most superficial part of the exudate, where there were rare gram-positive cocci. Cocci and bacilli were abundant in the colonic ulcerations, but a protracted search failed to reveal inclusion bodies in the lesions in the colon or in the remaining mucous membrane. There were neither ulcers nor inclusions in the stomach. The routine histologic sections of heart, aorta, lung, spleen, liver, pancreas, adrenal, kidney, prostate and testis contained no inclusions. The salivary glands were not examined. The final pathologic diagnosis was chronic ulcerative colitis, pelvic peritoneal abscess, bronchopneumonia and acute ulcers of the esophagus.

Case 2.—A 36 year old white man was admitted to the Long Island College Hospital March 17, 1942. He had been hospitalized four years before because of a spontaneous subarachnoid hemorrhage, from which he recovered without sequelae. Otherwise he had always been in good health. Three days before his final admission he had a sudden attack of severe abdominal pain which, although generalized, was somewhat more severe in the left lower quadrant. There was no associated nausea or vomiting. The pain abated somewhat from its original

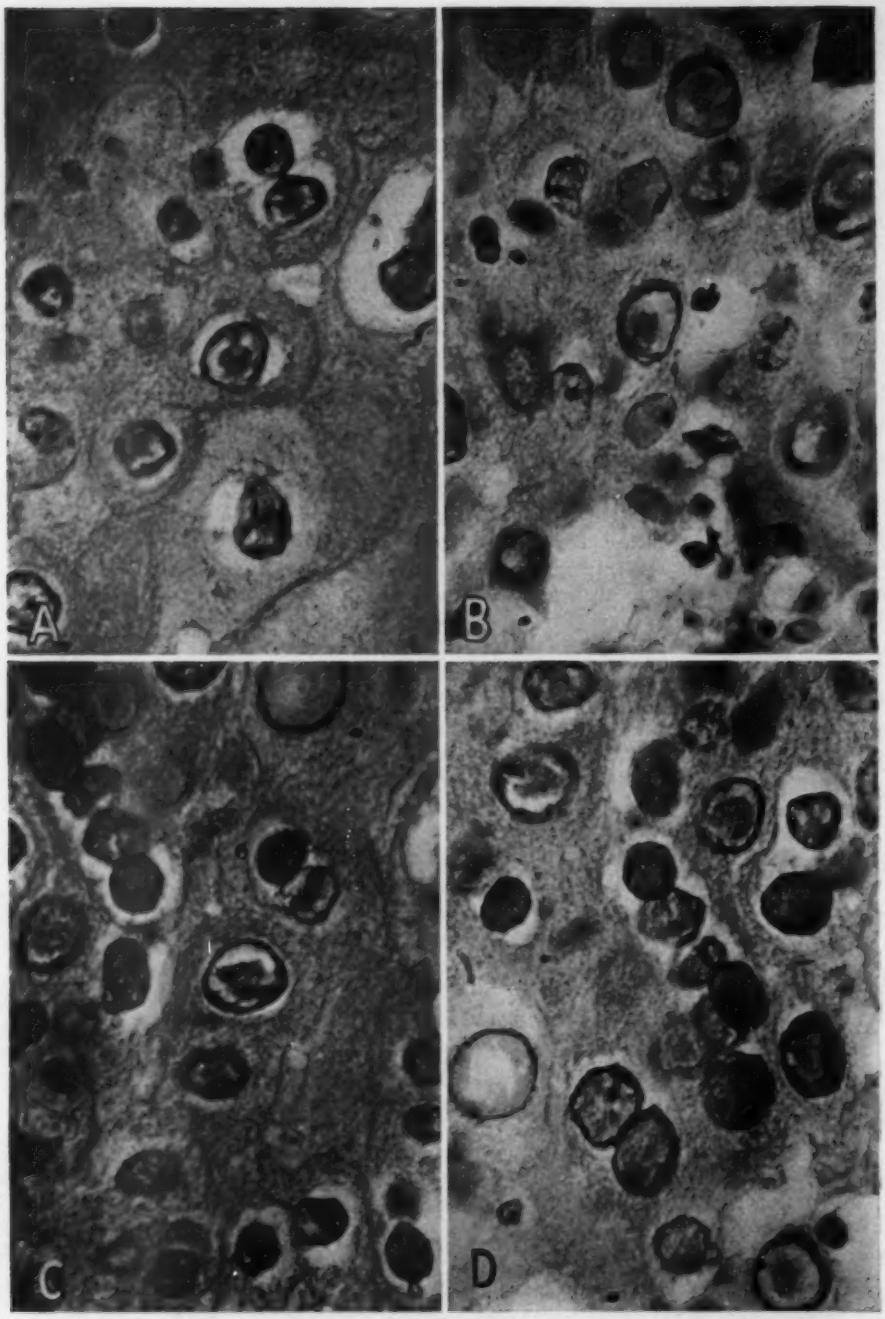

Fig. 2.—Intranuclear inclusion bodies in epithelial cells at the margins of ulcers. Hematoxylin and eosin; × 1,000. In *A* and *B* the central inclusion bodies with the surrounding pale zones and beaded nuclear membranes are readily seen. In *C* and *D* there are also many cells in which the inclusion appears to fill the entire space within the nucleus giving it a homogeneous appearance.

severity, but on the third day it again became extreme. He had chills and fever and was brought to the hospital. His temperature was 102.4 F. The pulse rate was 120. Abdominal tenderness and spasm were everywhere marked but were most pronounced in the left lower quadrant. There were 21,000 white blood cells per cubic millimeter of blood, and 90 per cent were polymorphonuclear leukocytes. The diagnosis of generalized peritonitis was made. Operation was thought to be contraindicated. He was treated conservatively and given sodium sulfathiazole (the sodium salt of 2-[paraaminobenzenesulfonamido]-thiazole) intravenously. He remained critically ill throughout the sixteen days of his life in the hospital. During the two days before death he passed several large bloody stools.

Autopsy was performed two hours post mortem. The intestines were bound together by fibrinous adhesions which when separated exposed several large and small pockets of gray purulent fluid. Approximately 800 cc. of purulent material lay beneath the dome of the diaphragm on the left and surrounded the spleen. Both the pus in the pockets between the intestinal coils and that in the subdiaphragmatic abscess contained the colon bacillus in pure culture.

The origin of the peritoneal infection was found to be an acutely inflamed and perforated diverticulum in the upper portion of the sigmoid colon. The perforation measured 8 mm. in diameter and communicated directly with the lumen of the intestine. In the necrotic margin of the eroded area lay two small branches of the left colic artery. Although at the time of autopsy these vessels contained thrombi, they were presumably the source of the bleeding into the gastrointestinal tract. An ulcer 1.5 cm. in diameter, with indurated and fibrous base and margins was situated on the lesser curvature of the stomach 4 cm. above the pylorus. There was no blood in the stomach, and no vessels could be found in the base of this peptic ulcer.

Four centimeters below the level of the cricoid cartilage, in the mucous membrane covering the posterior wall of the esophagus there was a superficial ulceration measuring 5 mm. in diameter. The erosion was covered by an abundant and elevated deposit of fibrin, which was easily scraped off.

Microscopically, this ulcer closely resembled those in case 1. Again it extended down to but not into the muscle, and again the degenerating epithelial cells at its margin contained acidophilic intranuclear inclusions lying in a clear space, around which the chromatin was concentrated at the nuclear membrane. Gram and Giemsa stains failed to reveal bacteria, yeasts or fungi. No inclusions were found elsewhere in the gastrointestinal tract or in any of the other viscera.

In this case the main part of the pathologic diagnosis was acute diverticulitis with perforation, generalized peritonitis, chronic peptic ulcer of stomach and acute ulcers of the esophagus.

Case 3.—A 49 year old white man came to the hospital June 18, 1942 because of fever and severe pain in the epigastrium and the right upper quadrant of the abdomen. On laparotomy he was found to have an acutely inflamed and distended gallbladder. The gallbladder was removed surgically, but the patient's temperature remained elevated, and shortly signs of acute generalized peritonitis developed. Death occurred on the tenth postoperative day.

The postmortem examination was made three hours later. In the abdominal cavity the omentum and the loops of the small intestine were bound tightly together and to the anterior abdominal wall by fibrous adhesions. When these adhesions were separated, numerous abscess cavities containing thick green mucoid fluid were found. Only the colon bacillus was cultured from this pus. The mesentery was twisted on itself and fixed by adhesions. The mesenteric veins

were thrombosed. The lower part of the jejunum and the entire ileum had a dark red to blue-black color. The intestinal wall here was edematous and easily torn. The serosa was covered by fibrin.

Scattered over the mucosal surface of the entire length of the esophagus were small superficial ulcerations, which measured 1 mm. to 3 mm. in diameter. These ulcers were rounded, and on their surfaces lay firmly adherent yellow fibrin.

Histologically, the esophageal lesions were similar to those in the preceding cases. The inflammatory reaction at the bases of the eroded areas, however, was more intense, and leukocytes were spread widely beneath the intact epithelium far from the periphery of the ulcer. In addition there were areas in the esophagus where the epithelial lining was thin and the cells were elongated and basophilic, giving the appearance of regeneration and the healing of earlier ulceration. The epithelial cells at the margins of the denuded areas contained intranuclear inclusion bodies which could not be distinguished from those in cases 1 and 2.

There were no other noteworthy anatomic alterations. The chief part of the pathologic diagnosis was acute cholecystitis, acute generalized peritonitis, volvulus, infarction of the small intestine and acute ulcers of the esophagus.

Case 4.—A 34 year old white woman was brought to the hospital Oct. 3, 1942 in the final stages of cachexia and starvation. Six months previously she had suddenly begun to have diarrhea, with passage of ten to fifteen watery stools daily, which frequently contained blood and mucus. In the early period of her illness she spent five weeks in another hospital, but her improvement there was so slight that she returned home, where she remained until her terminal admission. In the last two months of her illness the diarrhea diminished considerably, but nausea and anorexia persisted to such an extent that she was eating practically nothing. Extreme anasarca developed, which was thought to be the result of starvation and hemorrhage. The serum albumin was 1.3 Gm. and the serum globulin was 1.9 Gm. per hundred cubic centimeters. There were no amebas in the stools. The patient was obviously moribund on admission. Transfusion was of no avail, and she died nine days later without having been submitted to proctoscopy or other investigative procedure.

The postmorten examination was made four and one-half hours after death. The dependent subcutaneous tissues were so edematous that in some areas, such as the dorsa of the feet, actual fluctuation could be elicited. The peritoneal cavity contained 6,000 cc. of clear watery fluid, and in each pleural cavity there was 5,000 cc. of similar fluid. The colon was extensively ulcerated. In the cecum, the ascending and the transverse colon only shreds and polypoid masses of edematous mucosa remained. In the sigmoid the ulcers were numerous but discrete. The rectum was deep red, but its mucous membrane was intact.

The heart was small. The lungs were atelectatic, and in them there were scattered small areas of pneumonic consolidation. The liver contained much fat.

The lesion in the esophagus was almost identical with that seen in case 1. In the lower 5 cm. of the mucous membrane small erosions varying from barely visible pinpoint lesions to ulcers 3 mm. in diameter were scattered on the surrounding intact squamous epithelial surface. Slightly raised masses of dull yellow fibrin, which could be scraped away easily, covered the small denuded areas.

On microscopic examination the epithelial cells bordering on the ulcers contained intranuclear inclusion bodies identical with those seen in the preceding cases. Bacterial stains failed to reveal organisms. Again, as in the earlier cases, no inclusion bodies could be found in the epithelial cells of the remainder of the gastrointestinal tract or in any other organs.

The pathologic diagnosis was chronic ulcerative colitis, anasarca, fatty liver, bronchopneumonia and acute ulcers of the esophagus.

COMMENT

The histologic appearance of the intranuclear inclusion bodies around the lesion in these 4 cases is strong presumptive evidence of the viral origin of the esophageal ulcers. The inclusions and the cells containing them were so similar to those found in known virus infections that morphologically it was impossible to distinguish them. This is the type of inclusion which Cowdry [1] put in his class A, the group which is most certainly associated with viral infection. Of course, the observation of inclusion bodies does not in itself prove that a virus is the cause of the lesion in which they are seen, but with the exception of the experimental work of Olitsky and Harford,[2] in which similar appearing bodies followed subcutaneous injection of certain aluminum and ferric compounds into guinea pigs, there is no other demonstrated cause for their presence.

Both the gross and the histologic appearances of the esophageal lesions in the 4 cases were almost identical. In each the ulceration was superficial and extended for only a short distance beneath the previous limits of the epithelium. The infiltrating leukocytes in each case consisted predominantly of lymphocytes and large mononuclear leukocytes, while polymorphonuclear leukocytes were few and scattered. The type of intranuclear inclusion body in the epithelium bordering on the ulcer was identical in all 4 cases.

The distribution and extent of the lesion varied somewhat however. In cases 1 and 4 ulcers were numerous but were confined to the lower part of the esophagus. In case 2 the lesion was single and was in approximately the midportion of the viscus, while in case 3 many ulcers were scattered over the entire extent of the mucous membrane.

There is a striking similarity in all respects between cases 1 and 4. The duration and the course of the illness were the same in both. In both the condition clinically was typical nonspecific chronic ulcerative colitis, and the colonic and esophageal lesions in the 2 cases were almost identical as to character and disposition when seen at autopsy. The presence of the inclusion bodies in the esophageal epithelium of both of the patients of course suggests the possibility that the virus which presumably caused the erosions in the esophagus may have been responsible for the ulcers in the colon as well. Against this hypothesis is the failure to find inclusions in the remaining colonic epithelium, together with their presence in esophageal ulcers of 2 patients who,

1. Cowdry, E. V.: Arch. Path. **18**:527, 1934.

2. Olitsky, P. K., and Harford, C. G.: Am. J. Path. **13**:729, 1937; Proc Soc. Exper. Biol. & Med. **38**:92, 1938.

although they had intestinal lesions, did not present the picture of chronic ulcerative colitis. The latter of these two arguments against the etiologic significance of the inclusion bodies is the stronger since in several viral diseases although the lesions are widespread, only one type of tissue may contain inclusions. A notable example of this is vaccinia, in which Guarnieri bodies may appear in the stratified squamous epithelium of the cornea or the skin but not in the visceral lesions.

Patients 1 and 2 were admitted to the hospital within five days of each other and died on the same day. If the inclusions have no etiologic significance in regard to chronic ulcerative colitis, then the possibility arises from this chronologic coincidence that the 2 patients were infected from a common source or one from the other. However, the beds of these patients were in widely separated parts of the hospital, and each was cared for by a different group of orderlies and nurses. In each case the nature of the illness necessitated an extremly restricted diet, but the food for each patient was prepared in the same kitchen.

The infectious nature of the esophageal lesion is also suggested by the fact that a review of the autopsy material from the Long Island College Hospital failed to reveal any inclusion bodies in similarly situated ulcers observed in the past. It seems unlikely that 2 cases would appear simultaneously where none had occurred previously if the cause was not an infectious organism. Against the theory of contagion is the fact that the third patient did not enter the hospital until two and one-half months after the deaths of the first 2, and the fourth was admitted only after a further interval of two months.

It is obvious from the inconsequental nature of the esophageal ulcers that they played little part in the disease pictures presented by the patients. If the viral cause of these lesions is admitted, it seems most probable that the infection occurred incidentally and shortly before death. It is conceivable that each patient may have harbored the virus as a harmless saprophyte and that it became pathogenic only when the "resistance" of the host was lowered by the debility accompanying the end stages of a fatal disease. A similar explanation has been postulated for the spontaneous appearance of labial herpes during febrile illnesses.

Material from 3,300 autopsies at the Long Island College Hospital and from 1,500 autopsies at the Long Island Division of the Kings County Hospital included 38 instances of esophageal ulceration. In none of these could inclusion bodies be found in the lesions. The incidence of ulceration was in all likelihood much higher than these figures would indicate. Until interest was focused on the esophagus, small ulcers there were probably overlooked. It may be, too, that the inclusion bodies are present only for a short period in the initial stage of the disease and later disappear as the lesion increases in size and is complicated by secondary bacterial infection. An analogous disappearance of

inclusions in older lesions occurs in several virus diseases, notably vaccinia, in which the Guarnieri bodies are present only during the third twenty-four hours after inoculation, and in virus III disease, in which the intranuclear inclusions are absent in the advanced stages of inflammation.

A survey of the literature disclosed only 1 other case of esophageal ulceration in which inclusion-bearing cells were associated with the lesion. Hartz and van der Sar [3] reported the observation of a single superficial ulcer in the esophagus of a 48 year old Negro in the Netherland West Indies who died of pulmonary tuberculosis and syphilitic aortitis. Their case differs from those described here in that the nuclei containing the inclusions were those of fibroblasts in the granulation tissue and especially those of the intimal connective tissue cells of venules and capillaries. Inclusions did not occur in the epithelium of the esophagus. The photomicrographs of the inclusions also suggest that Hartz and van der Sar observed a quite dissimilar lesion.

Von Glahn and Pappenheimer [4] and Farber and Wohlbach [5] described cases in which on postmortem examination there were found in salivary glands and viscera cells whose nuclei contained typical viral inclusion bodies. These authors did not describe inclusion-bearing cells in the esophageal epithelium, although a detailed examination of material was made in each instance; nor did they mention inclusion bodies in this situation in their extensive discussion. It has not been possible to find in the literature any description of a lesion in the esophagus similar to that seen in these 4 cases.

SUMMARY

Four cases of esophageal ulceration in which intranuclear inclusion bodies resembling those seen in viral infections occurred were encountered in the same hospital during a short period. No similar lesions were found in a review of the earlier autopsy material, representing 4,800 necropsies and 38 cases of esophageal ulcers. The similarity of the inclusions to those known to be initiated by a virus and the sudden and almost simultaneous appearance of 2 of the 4 cases suggest an infectious origin. Although the esophageal ulcers were most probably the result of a terminal and incidental infection in a debilitated patient and have no relation to the primary disease, it is interesting that in 2 of the patients the primary disease was nonspecific chronic ulcerative colitis.

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3. Hartz, P. H., and van der Sar, A.: Genesk. tijdschr. v. Nederl.-Indië **81**:1310, 1941.

4. Von Glahn, W. C., and Pappenheimer, A. M.: Am. J. Path. **1**:445, 1925.

5. Farber, S., and Wohlbach, S. B.: Am. J. Path. **8**:123, 1932.

Case Reports

CARCINOID TUMOR OF THE GALLBLADDER

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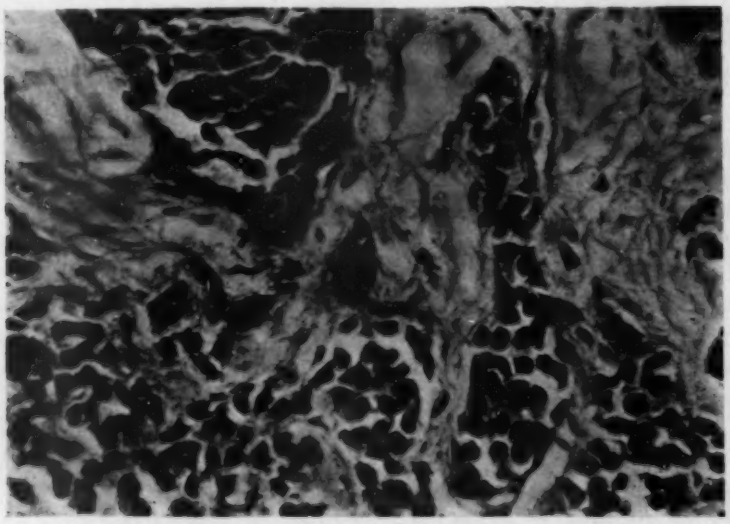
Carcinoid tumors have been found in a number of locations other than the appendix and the small intestine,[1] but a review of the literature reveals only 2 cases of carcinoid tumor of the gallbladder.[2] Both the patients were women, one 64 years old, the other 66 years old, and in each instance the tumor was an incidental finding at autopsy. Following is the report of a third case.



Photomicrograph of gallbladder showing the uniform small cells typical of carcinoid tumors; hematoxylin and eosin; × 660.

REPORT OF A CASE

A 66 year old white woman was admitted to the Western Pennsylvania Hospital, of Pittsburgh, complaining of weakness, general aches, vomiting at intervals and a loss of 40 pounds (18 Kg.) in weight over a nine month period. Roentgenograms following oral administration of dye failed to reveal a gallbladder shadow. Numerous other laboratory procedures gave no significant results.

Ten days after admission cholecystectomy was performed. The thirteenth day after the operation the patient was ambulatory but remained listless and was not

From the Western Pennsylvania Hospital Institute of Pathology (Ralph R. Mellon, M.D., director).

1. Ashworth, C. T., and Wallace, S. A.: Arch. Path. **32**:272, 1941.

2. Joel, W.: Centralbl. f. allg. Path. u. path. Anat. **46**:1, 1929. Porter, J. E., and Whelan, C. S.: Am. J. Cancer **36**:343, 1939.

discharged from the hospital until forty-three days after admission. About ten weeks later she died at home, allegedly of causes unrelated to the gallbladder.

The gallbladder removed at operation measured 15 cm. in length and 3.5 cm. in diameter. The serosa was smooth and glistening. The lumen was filled with many small stones 1 to 3 mm. in diameter and one larger stone, measuring 3.5 by 2.5 by 2.5 cm. The bile was greenish black; the mucosa was trabeculated and ulcerated, and the wall averaged 1 to 2 mm. in thickness. A circumscribed firm yellowish white nodule, 3 mm. in diameter, involved most of the thickness of the wall in the midfundus region, but was covered by both serosa and mucosa.

Examination of sections through the nodule showed small and large masses of fairly uniform, rather small cells with somewhat vacuolated, bluish pink–staining cytoplasm and poorly defined cell boundaries. The nuclei were darkly stained, and although somewhat variable in shape, were usually round or oval. The cell masses were surrounded by a moderate amount of collagenous stroma (figure). Some of the cell masses were separated by clear spaces from the surrounding stroma, and there was some tendency toward formation of clear spaces in the centers of cell masses. The nodule as a whole was not distinctly encapsulated, and cell masses were present just beneath the serosa. The cells showed occasional small argentaffin granules with silver staining and stained red with Masson's trichrome stain. The gallbladder otherwise showed moderate chronic inflammation.

The diagnosis was: carcinoid tumor of the gallbladder and chronic cholecystitis with cholelithiasis.

SUMMARY

A case of carcinoid tumor of the gallbladder in a 66 year old white woman is reported. The tumor was an incidental finding in a gallbladder which was filled with stones and showed chronic inflammatory change. This appears to be the third reported case of carcinoid tumor of the gallbladder.

OSTEOCHONDROBLASTIC MENINGIOMA OF THE LEFT CEREBELLAR HEMISPHERE

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Neuropathologist, Kings County Hospital
AND
BERNARD J. FICARRA, M.D.*
BROOKLYN

A 62 year old white man, a porter, was admitted to Kings County Hospital to the service of Dr. Jefferson Browder, Jan. 17, 1940. His illness began two and a half months before admission. At that time, while he was working on the docks, some one opened a door behind him, striking the right side of his head. He was dazed and dizzy for a short period afterward. He complained of a dull headache. Two or three days later he noticed that on standing he tended to fall to the right and that on walking he veered to the right. This and the inconstant but daily headache continued. For about six weeks before entering the hospital he complained of progressive weakness of the right leg. January 11, he became restless and confused, taking off and putting on his pajamas, talking with a mumbling speech and appearing quite dull. He was not drowsy but slept more than usual. He had not complained of visual disturbances, but the family noticed that in eating he had difficulty in locating his mouth.

He was well developed and well nourished but appeared ill. The temperature was 99 F.; the pulse rate, 90; the respiratory rate, 28; the blood pressure, 170 systolic and 120 diastolic. He was stuporous, pulled at the covers and removed his shirt. His movements were almost athetoid. He responded to simple commands.

Pupils were constricted, equal, regular and reacted to light. Extraocular movements could not be tested. There was doubtful blurring of the nasal half of the right disk. The teeth were carious; the pharynx, slightly hyperemic; the left tonsil, enlarged and cryptic. Moderate rigidity of the neck was present. The lungs were resonant; a few evanescent rales were heard at both bases. There was no cardiac enlargement; a systolic murmur was heard at the apex. All deep reflexes were present, although sluggish. The right ankle jerk was not obtained. Babinski's signs were not demonstrable. Lumbar puncture gave an initial pressure of 12 mm. of mercury. The cerebrospinal fluid was clear and colorless and contained 6 white cells per cubic millimeter. A Wassermann test of the spinal fluid and one of the blood were negative. Chemical examination of the blood revealed urea 40 mg., creatinine 1.24 mg. and sugar 200 mg. per hundred cubic centimeters. The urine was normal.

January 17, the left upper extremity seemed weaker than the right. The reflexes were more active on the right; the plantar reflexes were flexor in type. January 24, the patient became markedly ataxic and displayed overgroping in reaching for moving objects, with marked loss of check phenomena in the left upper extremity. When the arms were held in extension, the left drifted upward and outward; the right became flexed and dropped to his side. January 28, septic

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fever set in and rose in a few days to 104 F. The patient became unconscious, unable to respond to questioning or painful stimuli or pressure. Pneumonic consolidation of the lower lobe of the right lung occurred, and the patient died suddenly February 5.

January 24, encephalography showed the third ventricle in the midline with no shifting. No evidence of a fracture of the skull was seen. Roentgenograms

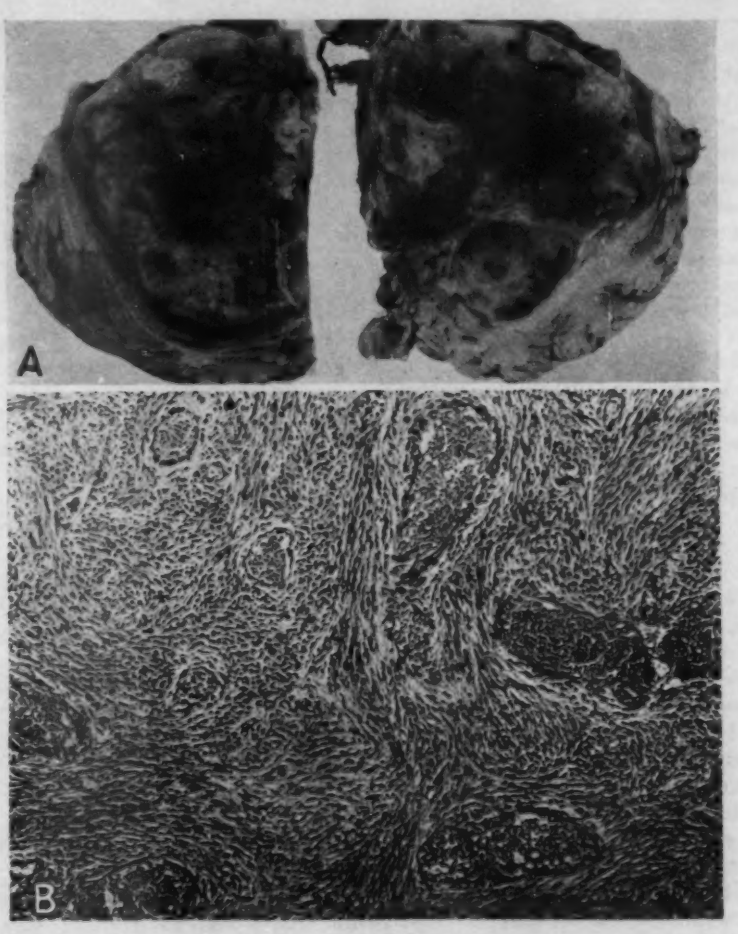

Fig. 1.—*A*, transverse section showing the size and the position of the tumor in the left cerebellar hemisphere. *B*, low power view of the fibroblastic and angioblastic structure of the tumor.

of the skull, January 29, showed air about the convolutions of the brain and a very small amount of air in the third ventricle and the basal cistern. Practically no air had entered the lateral ventricles. A large amount of air was seen in the subarachnoid space and in both parietotemporal regions, indicating some degree of atrophy of the brain. Further studies, January 30, demonstrated an outlined fourth ventricle but no filling of the third or of the lateral ventricles.

The autopsy revealed diffuse bronchopneumonia, chronic passive congestion of the liver, a duodenal ulcer, generalized arteriosclerosis, multiple retention cysts of the kidneys and a small solitary cyst in the tail of the pancreas. No evidence of fracture of the skull or of hematoma was found. The dura was normal except over the left lateral lobe of the cerebellum, where it was adherent to a tumor occupying two thirds of the left cerebellar hemisphere. The tumor was pearl-like and glistening, firm, and densely adherent to the underlying cerebellum within which it lay. Over it the dura mater was firmly attached and could not be separated. Between the cerebellum and the left occipital lobe there was a loose free mass, the size and appearance of a pearl, which was firm but not hard.

After fixation in 5 per cent solution of formaldehyde, the brain was studied more closely. Both frontal lobes showed marked atrophy. The vessels of the circle of Willis showed narrowing of their lumens due to calcification. The left cerebellar hemisphere was larger than the right. On the anterior surface a large pearly growth protruded above the surface of the left hemisphere. The cut surface presented a well encapsulated round mass, 4.5 by 4.5 cm. (fig. 1 *A*), grayish brown and firm. There were scattered pea-sized black areas. The cerebellar tissue formed a 1 cm. ring about the tumor. Section through the tumor revealed small pea-sized areas which were bony-hard and calcific. The tumor did not press on the pons or the medulla. The ventricular system was uniformly dilated. Pinhead-sized cysts were found in the choroid plexus. From the standpoint of histology, the essential features of the tumor were areas of fibroblastic and mesothelial cellular tissue (fig. 1 *B*) with interspersed cartilage and adult bone. There were scattered circumscribed areas of cords of flat cells, some of which had a whorl-like arrangement with small blood channels in their centers (fig. 2 *A*), not unlike the leptomeningioma of arachnoid type described by Globus.[1] Endothelial cells and blood vessels of variable number and in different stages of differentiation were noted. The most striking tissue was new bone in all stages of formation, both membranous, in a matrix of fibroblastic tissue, and cartilaginous.

Some areas showed a transition from fibroblastic tissue to cartilage and then to bone. Many multinuclear giantlike cells were present near capillaries surrounding foci of bone. An occasional giant cell nested in a concavity along the edge of the osseous tissue (fig. 3 *B* and *C*). No psammomma bodies were found. There was a small amount of calcareous deposit about some blood vessels.

In view of the short history one could exclude the possibility of a cerebellar hemorrhage with secondary calcification and bone formation. Moreover, there was no evidence of hemorrhage, of pigment or of scar formation. As to teratoma, Ewing[2] found only two reports, one by Saxer and the other by Eberth. Saxer found a complex teratoid tumor containing cartilage, bone, muscle, chordal tissue, glandular alveoli, cysts lined by cylindric, ciliated or squamous epithelium, pigmented retinal epithelium, choroid plexus, fetal brain tissue and ganglions. Eberth described a tumor, connected by a pedicle with the dura, which was composed of fat, muscle, lymphoid tissue and nerves. In our tumor only osteoid tissue, cartilage and proliferating vascular elements were found.

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1. Globus, J. H.: A. Research Nerv. & Ment. Dis., Proc. (1935) **16**:210, 1937.

2. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1940.

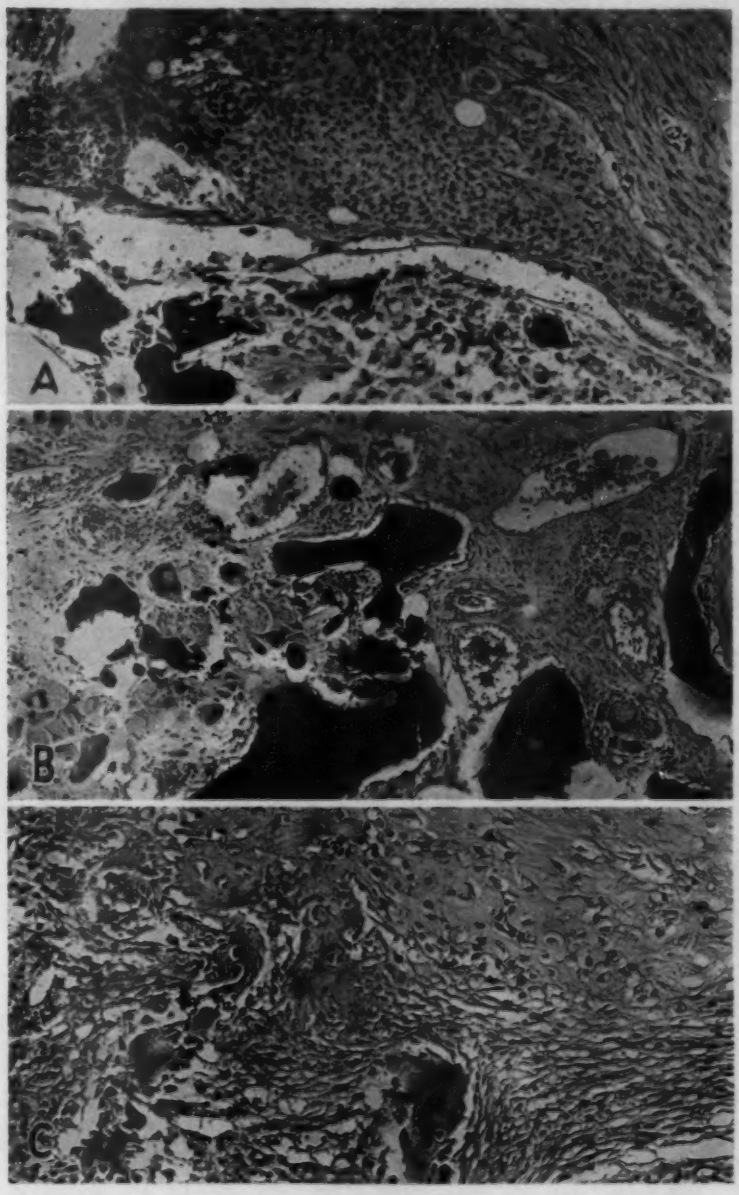

Fig. 2.—*A*, area of whorl-like cell groups. The lower half of the photomicrograph illustrates osteoblastic tissue. Hematoxylin and eosin; × 100. *B*, numerous areas of bone and capillary formation with interspersed multinuclear giantlike cells. Calcium is deposited about the blood vessels. Hematoxylin and eosin; × 100. *C*, photomicrograph demonstrating the transition from fibroblastic tissue to cartilage and then to bone. Hematoxylin and eosin; × 100.

Tumors attached to the dura generally originate from the dura or from the arachnoid. Bailey and Bucy,[3a] Globus [1] and others have demonstrated the multipotentiality of tumors of meningeal origin, which was also fully recognized by Cushing and Eisenhardt.[4] Alpers [5] reported a tumor diagnosed as osteochondroma which he believed arose from a fibroblastic tumor of meningeal origin. He described two additional tumors attached to the choroid plexus. Wolf and Echlin [6] reported a tumor diagnosed as osteochondroma which was attached to the falx and which in their opinion was not meningeal in origin.

Our tumor was not unlike the reported ones, but it contained an area characteristic of meningioma, which establishes beyond doubt its meningeal origin.

The source of the bone and the cartilage in these tumors has been the topic of much dispute. That bone may develop in the meninges is borne out by the development of bony plaques in the dura and the falx, with all the characteristics of true bone. This is established further embryologically by Globus, who demonstrated the development of meninges next to primitive mesenchyme prior to the separation of bone and periosteum from the meninges. That cartilage as well may form under these circumstances is the opinion of Alpers [5] (and perhaps others). Although Bailey [3b] denied this possibility, it is based on fact that membranous bones of the skull do not lay down cartilage. If it is accepted that the osteoblastic tumors attached to the dura are meningeal in origin, it must be accepted that cartilage is a part of the same tumor. We believe that in our case the development of bony islands and cartilaginous areas could be traced directly to the fibroblastic tissue.

This tumor, therefore, was one of mixed type exhibiting some of the histologic characteristics of meningioma. It manifested the multipotentiality of the tumors of this class, as demonstrated by its fibroblastic, angioblastic, osteoblastic and chondroblastic features. The last two elements are quite unusual. Apparently, the tumor was of slow growth and not related to trauma. The slow growth probably accounted for the absence of significant symptoms, which is explained by the ability of the brain to accommodate itself to slowly developing pressure.

While tumors of the brain are notorious for the frequency with which they produce atypical signs, those of the posterior fossa usually conform to a somewhat normal pattern. The patient with a tumor of the posterior fossa presents evidence of increased intracranial pressure. Elevation of the spinal fluid pressure and papilledema are almost constant. Grant and associates [7] studied 158 cases of cerebellar tumor which presented unusual symptoms. In this series only 15 cases failed to show papilledema. Lumbar puncture in our case gave normal results. The roentgenogram revealed nothing to suggest a lesion in the posterior fossa.

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3. (*a*) Bailey, P., and Bucy, P. C.: Am. J. Cancer **15**:15, 1931. (*b*) Bailey, O. T.: Arch. Path. **30**:42, 1940.

4. Cushing, H., and Eisenhardt, L.: Meningiomas, Springfield, Ill., Charles C Thomas, Publisher, 1938.

5. Alpers, B. J.: Ann. Surg. **101**:27, 1935.

6. Wolf, A., and Echlin, F.: Bull. Neurol. Inst. New York **5**:515, 1936.

7. Grant, F. C.; Webster, J. E., and Weinberger, L. M.: Am. J. M. Sc. **202**:313, 1941.

The history of trauma, the fluctuating clinical picture, the absence of any characteristic symptoms with few localizing signs, associated with stupor, pointed to a lesion of traumatic origin. The patient presented difficulties in "locating his mouth," which is generally a symptom of cerebral origin. The results of the study of the encephalograms were likewise misleading and failed to demonstrate the presence of the tumor.

The salient, unusual feature of this case was the presence of a large tumor of the cerebellum with no increase in the intraspinal pressure and with inconstant cerebellar symptoms.

SUMMARY

A case is reported of a tumor of the cerebellum, which did not present papilledema or other clinical signs usually associated with a tumor of the posterior fossa. The tumor was diagnosed as meningioma with osteoblastic, chondroblastic and angioblastic features.

TUMOR OF THE CAROTID BODY AND THE PANCREAS

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In 1891 Marchand first described a tumor of the carotid body. Since that time approximately 275 examples of this type of neoplasms have been reported. The literature has been reviewed by Bevan and McCarthy,[1] Rankin and Wellbrook,[2] Greene and Greene [3] and Peterson and Meeker.[4]

In all the reported instances of tumor of the carotid body the neoplasm has been localized in the neck, never spreading beyond the lymph nodes in the immediate neighborhood of the primary mass. The case reported by Gilford and Davis [5] and that by Mönckeberg [6] are the only ones on record in which autopsy disclosed a tumor outside of the neck. In the former the liver was studded with soft white nodules, and in the latter ovarian tumors were found. In both instances the diagnosis of tumor of the carotid body is doubtful.

In several reported cases both carotid bodies have been involved, either simultaneously or one after the other. One autopsy [7] showed, in addition to the neoplasm of the carotid body, tumors of the organs of Zuckerkandl. These organs are two small bodies of chromaffin tissue which lie on the anterior surface of the abdominal aorta at the origin of the inferior mesenteric artery. They are conspicuous in fetuses from 5 months to full term, after which they rapidly undergo atrophy. From the second year of life on, they are extremely difficult to find.

The present report records observations on a patient with a tumor of the carotid body in whom a nodule histologically identical with the mass in the neck was present in the body of the pancreas.

REPORT OF A CASE

A 47 year old Negro entered Barnes Hospital in 1927 for treatment of a mass in the left side of the neck. The mass had been present for five years, and in the few months prior to his admission to the hospital had increased in size. The Wassermann reaction of the blood was 4 plus, and a history of a primary syphilitic lesion occurring twenty-five years before was elicited. The mass, which did not pulsate, was considered to be a gumma, and the patient was given antisyphilitic treatment, which he discontinued after two months. For the following fifteen years he felt well, and the mass in his neck, although increasing constantly in size, caused him no discomfort.

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May 7, 1942, he was readmitted to Barnes Hospital. He was now 62 years of age. He gave a history of dyspnea, orthopnea, paroxysmal nocturnal dyspnea, edema of the ankles and enlargement of the abdomen, all of fourteen months' duration. Sharp substernal pain was also present. He had been treated in another hospital twice during the past year for the same complaints, with slight improvement following rest in bed and digitalis therapy. The mass in his neck had now been present for twenty years and caused no discomfort. It was twice as large as it had been fifteen years before, and measured 12 cm. in diameter.

On admission the patient was moderately dyspneic and orthopneic. There was edema of the ankles and over the sacrum. The veins of the neck were distended. The pupils were small and reacted sluggishly to light. There was a firm mass, 12 cm. in diameter, below the angle of the left mandible. It was freely movable laterally but fixed vertically. This mass was thought to have expansile pulsation and was considered to be an aneurysm of the left carotid artery. The heart was enlarged, the apex impulse being seen and felt in the anterior axillary line. There was a soft, short systolic murmur at the apex. The aortic second sound was hollow, high pitched and loud, with a short diastolic murmur heard best in the second right intercostal space. The blood pressure in each arm was 170 systolic and 70 diastolic. The liver was enlarged and slightly tender. The peripheral arteries were moderately thickened. The knee jerks and ankle jerks were absent; otherwise the neurologic examination showed nothing abnormal.

There were 4,470,000 erythrocytes and 7,100 leukocytes per cubic millimeter of blood. The hemoglobin level was 70 per cent. The differential percentages were: stab cells, 6; segmented neutrophils, 66; lymphocytes, 25, and monocytes, 3. The urine contained a trace of albumin. The Wassermann reaction of the blood was 4 plus. The nonprotein nitrogen of the blood amounted to 23 mg. per hundred cubic centimeters. Examination of the spinal fluid by lumbar puncture revealed normal pressure and dynamics. The Pandy test and the Wassermann test of the fluid were positive. The colloidal gold curve showed a midzonal reaction, and the total protein of the fluid was 47 mg. per hundred cubic centimeters. Kymographic roentgen examinations revealed the heart to be moderately enlarged. There was an increase in the excursions, especially over the right side of the heart, and the aorta filled and emptied rapidly. These findings were interpreted as indicative of aortic regurgitation.

Treatment with digitalis and diuretics resulted in no improvement in the condition of the patient. He began to complain of severe headaches, nausea and vomiting. No explanation for these symptoms was found. On the eighteenth day in the hospital, the pulse rate suddenly fell to 40 per minute, and an electrocardiogram revealed auricular fibrillation and complete heart block. This condition persisted until death. Ten days later, the temperature rose to 39.3 C. (102.7 F.), and the patient became semicomatose. The breath had a uremic odor. Fluids were administered parenterally, and feeding was begun by stomach tube. Severe diarrhea developed which persisted until death. He was comatose for the last twenty-eight hours of life. At this time the blood nonprotein nitrogen was 210 mg. per hundred cubic centimeters. The patient died June 7, 1942, thirty-three days after his admission.

Autopsy (twelve hours post mortem).—The heart was large, weighing 680 Gm. There was advanced syphilitic involvement of the ascending aorta with widening of the commissures of the aortic valve. There was moderate chronic passive congestion of the lungs, the liver and the spleen. The lower lobes of the lungs

were partially consolidated, and there was purulent bronchitis of all the lobes. The wall of the ileum was congested, and the lymphoid tissue of the colon was prominent. Other findings were moderate sclerosis of the aorta and coronary arteries, slight sclerosis of the hepatic, splenic and renal arteries, focal scars of the kidneys and calcification of the renal pyramids. The gastrointestinal tract, the gallbladder, the urinary bladder and the prostate gland were normal.

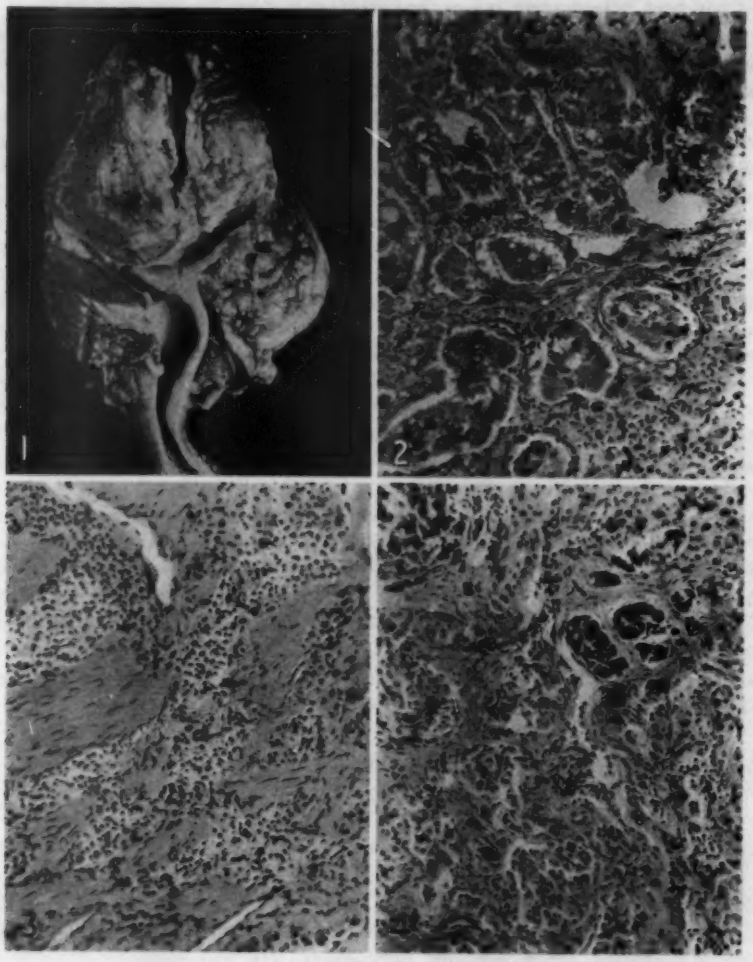

Fig. 1.—Cross section of the tumor of the carotid body. The common, internal and external carotid arteries are surrounded by tumor (⅔ original size).

Fig. 2.—A section of the tumor of the carotid body showing the alveolar arrangement of the tumor cells. × 98.

Fig. 3.—Wide bands of partially hyalinized fibrous tissue traverse the tumor of the carotid body. × 98.

Fig. 4.—A section of the nodule of tumor in the pancreas. Pancreatic acini are present among the tumor cells. × 98.

At the bifurcation of the left common carotid artery there was a firm nodular gray-pink mass, measuring approximately 10 cm. in diameter. This mass completely surrounded the internal and external carotid arteries, which were patent but compressed (fig. 1). The cut surface of the tumor was gray-pink and traversed by fine fibrous interlacing trabeculae. The mass was attached only to the carotid arteries, being easily separated from surrounding structures in the neck. There were no nodules of tumor elsewhere in the neck. The cervical lymph nodes were normal in all respects.

In the anterior part of the body of the pancreas, which was otherwise normal, there was a firm spherical gray-pink nodule, measuring 15 mm. in diameter. This mass was not well demarcated from the pancreatic tissue. It was within the substance of the pancreas and not visible on the surface.

Microscopically, the tumor of the neck was composed of moderately large cells with eosinophilic cytoplasm and fairly large vesicular nuclei, which showed moderate variation in size and shape. Most of the nuclei contained one or more prominent nucleoli, and the chromatin was scattered throughout the nucleus in the form of granules. Mitotic figures and multinucleated cells were present in moderate numbers. The tumor cells were arranged in alveolar pattern, supported by fine strands of collagen (fig. 2). Numerous large sinusoids coursed through the tissue, in many areas in close relation to the tumor cells. At no point were there tumor cells within the sinusoids. A moderate number of macrophages containing irregular granules of golden brown pigment was present. There were numerous accumulations of lymphocytes and plasma cells in the tumor. Large broad bands of fibrous tissue divided the mass into large nodules. Much of the connective tissue showed hyaline change (fig. 3). There were no enlarged lymph nodes in the neck, and no evidence of tumor involvement of lymph nodes was found. The tumor in the pancreas was microscopically identical with that in the neck. At one point there was pancreatic tissue within the tumor nodule (fig. 4).

Tissue from each mass of tumor, fixed in solution of formaldehyde, was stained by Schmorl's method[8] in an attempt to identify chromaffin granules. None were found in either tumor. The only other fixative used was a modification of Helly's fluid in which zinc chloride was used instead of mercuric chloride. This tissue had been in alcohol, which made it unfavorable for demonstration of chromaffinity, and although it was stained by Wiesel's technic,[8] no granules were demonstrable.

COMMENT

In view of the advanced syphilitic aortitis, the dilatation of the ring of the aortic valve, the enlargement of the heart and the congestion of the lungs, the liver and the spleen, the immediate cause of death was obviously cardiac failure. The history of cardiac decompensation for the year before death bears out this conclusion. Failure to improve on treatment with digitalis and diuretics is the rule in syphilitic aortic valvular disease with regurgitation, and it was true in this case.

The tumor of the left carotid body and that of the pancreas were incidental findings. That in the neck had been present for twenty years. The duration of the mass in the pancreas is, of course, unknown.

The tumor in the neck was grossly and microscopically characteristic of tumor of the carotid body as that condition has been described in the

8. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, pp. 267-268.

literature. The tumor in the pancreas was histologically identical with the mass in the neck. Since such a tumor has not been hitherto described in the pancreas, a consideration of the relation of the two neoplasms must necessarily be inconclusive.

The tumor in the pancreas must be either a metastasis from the mass in the neck or a separate neoplasm illustrating multicentric origin of neoplasm in similar tissues. There is normally no tissue in the pancreas which is similar in structure to the carotid body, from which the tumor in the pancreas might have arisen. However, it is well recognized that there is an abundance of chromaffin tissue along the abdominal aorta. It is possible that the tumor in the pancreas was derived from ectopic chromaffin tissue which had at some time become incorporated in the pancreas.

Tumors which spread widely occasionally metastasize to the pancreas, although this is not especially common. No neoplasm of the carotid body has ever been known to spread beyond the lymph nodes of the neck. In this particular instance, even these lymph nodes were uninvolved. It is therefore extremely unlikely that the nodule in the pancreas is a metastasis from the tumor of the carotid body. The idea of multicentric origin of neoplasm most satisfactorily explains the origin of the tumor of the pancreas.

The failure to demonstrate the presence of chromaffin granules in both tumors was not unusual. The fixation of the tissues was not optimal for the preservation of these granules. In those instances in which studies of chromaffinity have been carried out, there have been as many negative as positive results. It must be concluded that the chromaffin property of this type of tumor is an inconstant one.

Tumor of the carotid body has been studied by others for the presence of epinephrine, and in no instance was there a positive reaction. This is in agreement with the clinical findings, in that no disturbances have been observed in patients with this tumor, other than those directly related to the size of the mass or to the diminution in the supply of blood to the brain on the involved side. No relation exists, therefore, between the presence of chromaffin granules and that of epinephrine in these tissues.

Consideration of the incidence of tumor of the carotid body throws little light on the cause of the lesion. The patients have been between 7 and 73 years of age, the majority being between 40 and 60 years. They are about equally divided between the two sexes. No racial preponderance has been observed. Occurrence of the tumor in Negroes has been described. The average duration of the tumor when first seen by a physician is about seven years. In some cases that have been described the duration was thirty-seven years.[6] A study of the literature reveals no relation to any other recognized disease.

Numerous names have been applied to the tumor arising in the carotid body, among them being "perithelioma," "endothelioma," "paraganglioma," "ganglioneuroma," "angioma," "angiosarcoma," "neuroblastoma," "carcinoid," "sympathetic nevus," "carotid tumor" and "chromaffinoma." A tumor is properly named on the basis of the cell of origin rather than on that of the organ of origin or that of the structure of the tumor. The carotid body is generally believed to arise from the sympathogonia, cells of ectodermal origin, which are considered

to be the precursors of the sympathetic and chromaffin systems. The small masses of chromaffin tissue which are scattered along the abdominal aorta, and also the carotid body, are called paraganglions. The cells of which they are composed are more properly called chromaffin cells, and a tumor of any of these masses should be called a chromaffinoma. It is of interest to note that the "Standard Nomenclature of Disease" recognizes only the names of "benign or malignant tumor of the carotid body."

SUMMARY

A tumor of the carotid body associated with a histologically identical tumor in the pancreas is reported. The question of metastasis or multicentric origin is discussed. The various terms by which these tumors are known are mentioned, and "chromaffinoma" is selected as the most appropriate.

General Reviews

PERMEABILITY OF THE BLOOD-BRAIN BARRIER TO NEUROTROPIC VIRUSES

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In a recent review of the literature on the problem of the blood-brain barrier I arrived at the following conclusions: 1. The term "blood-brain barrier" expresses a selective permeability of the capillaries of the central nervous system. 2. The ability of aniline dyes, toxins, antibodies and drugs to pass the walls of these capillaries is determined by the electrical charge on each of these substances. 3. The capillaries of the central nervous system are permeable to electropositive and electroneutral substances but impermeable to electronegative ones.

In view of the almost unlimited number of substances to be considered, the permeability of the blood-brain barrier to neurotropic viruses was discussed only in a condensed form. The practical importance of the subject, as well as the large number of interesting observations pertaining to it, justifies a more extensive review of the literature. Moreover, it will be seen presently that opinions concerning the permeability of the blood-brain barrier to neurotropic viruses are still widely divergent. In an excellent article on neurotropic viruses Hurst expressed himself as follows: "The limited evidence available suggests that viruses do not easily pass the barrier." In a subsequent passage of the same article he was even less reserved when he wrote: "From a pathogenetic point of view the significance of virus in the blood lies not in the immediate consequences, for the normal haematoencephalic barrier is impervious to most if not all viruses." An entirely different view has been taken by Doerr, who emphatically discarded the concept that the capillaries of the central nervous system are impermeable to neurotropic viruses.

The fact that two authors equally conversant with the subject arrive at conclusions so widely divergent raises the question whether the criteria for permeability of the blood-brain barrier to neurotropic viruses have always been visualized with sufficient clarity. It is now the consensus that the majority of neurotropic viruses, if not all of them, are able to reach the central nervous system along neural pathways. This fact alone, however, cannot be considered as evidence that they are unable

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to pass through the capillaries of the central nervous system. In the first place it must be shown that the neural pathways are the only routes by which neurotropic viruses reach the central nervous system. In the second place it must be ascertained whether or not virus injected into some peripheral tissue reaches the capillaries of the central nervous system through the circulation.

In that respect neurotropic viruses fall into two distinct groups. Examples of those belonging to the first group are the viruses of pseudorabies, louping ill, equine encephalomyelitis and St. Louis encephalitis. These viruses are not strictly neurotropic. They invade the blood stream and may circulate for several days. If, nevertheless, they can be shown to reach the central nervous system exclusively along neural pathways, it may safely be assumed that they are unable to pass through the capillaries of the central nervous system.

The problem is more complicated in the case of the strictly neurotropic viruses, such as the viruses of poliomyelitis, rabies and Borna disease. These viruses when injected into an extraneural tissue do not invade the blood stream in any appreciable amounts. Experiments of this type, therefore, give no clue as to whether or not these viruses are able to pass the capillaries of the central nervous system. The only possibility of obtaining an answer to this question is given by the intravenous injection of large amounts of these viruses. If the injected virus fails to infect the experimental animal by the intravenous route, its inability to pass the capillaries of the central nervous system may be considered as proved. If infection takes place, several possibilities must be taken into consideration. The central nervous system may be invaded either directly through the capillary walls or the virus may leave the vascular system and enter nerve endings in some extraneural tissue. In the first case further experiments are necessary to determine whether the permeation is due to physicochemical forces or to multiplication within the capillary endothelium. The decision as to whether or not the capillaries of the central nervous system are permeable to an individual neurotropic virus therefore requires an answer to the following questions:

1. Is the virus able to reach the central nervous system along neural pathways?
2. Does it reach the central nervous system exclusively by this route?
3. Does the virus invade the blood stream?
4. If not, does it reach the central nervous system after intravenous injection?
5. If it reaches the central nervous system by the intravenous route, does it pass the capillaries of the central nervous system or does it first leave the vascular system and reach the central nervous system indirectly along neural pathways?

6. Where direct passage through the capillary walls can be demonstrated, the mechanism of permeation must be further investigated.

In the following sections the literature on neurotropic viruses will be reviewed from these points of view. It will be seen that each virus must be studied on its own merits. There is considerable variation in the mechanisms by which the individual neurotropic viruses reach the central nervous system.[1] Part of the diversity of opinion concerning the permeability of the blood-brain barrier is apparently due to the circumstance that findings holding true for one particular virus have been generalized for other viruses.

The question may be asked whether it is worth while carrying out all these complicated investigations only to determine whether viruses are able to pass the blood-brain barrier. Particularly in the case of the strict neurotropic viruses it may be argued that their transmission by neural pathways is all one needs to know concerning their penetration to the central nervous system. Actually, however, the investigations listed are indispensable if one wants information concerning the factors that determine the ability or the inability of neurotropic viruses to pass the blood-brain barrier. What those factors may be will be discussed in the second part of this review.

PERMEABILITY OF THE BLOOD-BRAIN BARRIER TO INDIVIDUAL NEUROTROPIC VIRUSES

Poliomyelitis.—In recent years the clear picture of the genesis of poliomyelitis as it revealed itself in experiments on Macacus rhesus has undergone some modifications. It has become quite doubtful whether the nasal mucous membrane can be considered as the portal of entry in human beings. An ever increasing number of investigators are inclined to attach more importance to the intestinal route. The cynomolgus monkey and the green African monkey have been shown to be susceptible to this mode of infection. Moreover, it will be seen in later paragraphs that the manner in which virus spreads in the animal body may be different for individual strains of the virus of poliomyelitis. At present it is difficult to judge whether these findings have any bearing on the question of the permeability of the blood-brain barrier to the virus of poliomyelitis. The following discussion will be confined to experiments in the monkey (M. rhesus) and to strains passed for a considerable time through this species.

It has been shown beyond doubt that the virus of poliomyelitis reaches the central nervous system by neural pathways. Flexner and Lewis, Landsteiner and Levaditi, Leiner and von Wiesner (1910 c) and many

1. Moreover, the species and the age of the experimental animal are important factors.

others have found that monkeys can be infected by the intranasal route. Three or four days after inoculation the virus was found exclusively in the olfactory bulbs and tracts (Levaditi and Landsteiner; Flexner and Clark; Sabin and Olitsky, 1938; Faber and Gebhardt). The same observation was made by Sabin and Olitsky (1936-1937) with the aid of histologic methods. Killing the experimental animals on successive days after inoculation of the virus and studying the pathologic changes of the central nervous system, Fairbrother and Hurst, as well as Faber, reached the conclusion that from the olfactory region the virus spreads to the hypothalamus, to the thalamus and, through the spinothalamic tract, to the lumbar cord. The spread of the virus within the central nervous system has recently been studied in greater detail by Howe and Bodian. The interested reader may be referred to their excellent monograph. The spread of the virus in these anatomic structures shows clearly that the virus after intranasal inoculation reaches the central nervous system neither through the circulation nor the cerebrospinal fluid. This conclusion is further substantiated by the fact that monkeys subsequent to removal or destruction of the olfactory bulbs or tracts can no longer be infected by the intranasal route. According to Armstrong and Harrsion, Schultz and Gebhardt (1936) and Sabin, Olitsky and Cox, instillation of aluminum sulfate, nitrophenol, trinitrocresol, mercurochrome or zinc sulfate has the same inhibitory effect.

Flexner and Lewis, Leiner and von Wiesner (1910 c) and Hurst produced poliomyelitis by injecting the virus into the sciatic nerve. Since Horster and Whitman showed that fluids injected into the nerve may reach the cerebrospinal fluid through artificial channels, it is questionable whether the experiments of the aforementioned authors are entirely conclusive in demonstrating the migration of the virus in the sciatic nerve. Recently Bodian and Howe reported interesting experiments which meet this objection. They were able to produce poliomyelitis by dipping the central end of the cut sciatic nerve into suspensions of the virus. They further made the important observation that the result was negative after the axis-cylinders had been destroyed by local application of solid carbon dioxide. According to Toomey, the virus of poliomyelitis is transmitted to the central nervous system essentially through the unmyelinated fibers of the sympathetic nervous system.

It is more than questionable, however, whether the virus after peripheral inoculation reaches the blood in any appreciable amounts. It has never been found in the blood of patients with poliomyelitis or in that of monkeys after intranasal instillation. Even after intracerebral injection it could be identified in the blood only occasionally (Gordon and Lennette).

Whether the virus of poliomyelitis reaches the central nervous system from the blood, therefore, can be decided only by experiments

in which it is injected by the intravenous route. Clark, Fraser and Amoss have shown that monkeys cannot be infected by this route unless overwhelming doses are given. This is significant since even seventy-two hours after intravenous injection the virus was still found in the blood.

Clark, Fraser and Amoss obtained positive results with repeated intravenous injections of large doses of the virus (approximately 1,250 intracerebral minimal lethal doses). Even under these conditions, however, the virus did not reach the central nervous system directly by way of the circulation. Lennette and Hudson (1935) and Armstrong showed that monkeys cannot be infected by the intravenous injection of the largest doses after section of the olfactory tracts or chemical treatment of the nasal mucous membrane. These results can be interpreted only on the assumption that the virus after intravenous injection is excreted on the nasal mucous membrane where it is taken up by the olfactory nerve endings. On the other hand, Lennette and Hudson (1936) reported that the intravenous injection of even relatively small and otherwise ineffective doses of virus produced poliomyelitis when the cerebral vessels had been damaged by an intracerebral injection of a sterile solution of starch. Therefore the experimental evidence shows convincingly that the virus of poliomyelitis is unable to pass the normal capillaries of the central nervous system.[2]

Rabies.—In its specific affinity for nerve tissue the virus of rabies closely resembles the virus of poliomyelitis. As early as 1887 di Vestea and Zagari showed in a series of classic experiments that the virus of rabies reaches the central nervous system by way of the peripheral nerves. They injected the virus into the sciatic and median nerves, respectively, and observed that paralysis started in the legs innervated by the injected nerves. Moreover, after intrasciatic inoculation the virus first appeared in the lumbar cord, whereas after injection into the median nerve the first appearance was in the cervical cord. Transection of the dorsal cord confined the virus to the part first reached from the injected nerve. Helman injected the virus into the tip of the tail. If the tail was removed within twenty-four hours after the injection, the animal remained in good health. Schweinburg and Windholz in experiments on parabiotic rats found that rabies developed only in the rats into which the virus had been directly injected. All these

2. More recent investigations seem to indicate that the genesis of the experimental disease may be different for various strains of the virus of poliomyelitis. German and Trask found a dermotropic strain highly infective by the intravenous route, even after section of the olfactory nerves. Jungeblut, Finer and Sanders reported that their cavian strain infected guinea pigs as easily after intravenous as after intracerebral injection.

experiments leave no doubt that after injection into a nerve or subcutaneous tissue the virus reaches the central nervous system by the neural route.

In view of the strictly neurotropic character of the virus, however, it can hardly be assumed that after subcutaneous injection appreciable amounts reach the blood. In the course of the experimental disease it has been found in the blood or organs other than the brain only exceptionally, and the rare positive findings may possibly be explained by centrifugal transmission of the virus along the nerves (Bertarelli; Remlinger and Bailly). After injection into the subcutaneous tissue the virus therefore has no opportunity of reaching the capillaries of the central nervous system.

Pasteur reported that animals can be infected with the virus of rabies by the intravenous route. The results of subsequent investigators were conflicting. Recent experiments of Remlinger and Bailly, Schweinburg, Panisset and Deschamps, and Hurst, however, leave no doubt that with sufficiently virulent strains and adequate dosage the intravenous injection leads to positive results in a considerable proportion of animals.

Whether the virus after intravenous injection reaches the central nervous system directly by way of the capillaries is a much debated question. Hurst found that removal of the olfactory lobes did not prevent infection by the intravenous route. Since, however, the virus may reach the central nervous system by any peripheral nerve, this negative result is obviously of little significance. Thus far no crucial experiments have been reported which would answer the question definitely. Some pertinent observations, however, may be mentioned. The fact that many workers obtained negative results with intravenous injections and the further fact that even after inoculation of the largest doses of the most virulent strains not all of the animals had rabies is difficult to reconcile with the assumption that the virus reaches the central nervous system directly from the blood stream. Moreover, a comparison of the incubation periods following intracerebral and intravenous injection of the virus (Schweinburg, 1932) tends to corroborate one's doubts. With the first route the average period of incubation in three experiments was nine and three-tenths days; with the second route in five experiments it was thirty-one and four-tenths days. This significant difference also would be difficult to understand if the virus reached the central nervous system directly from the blood, whereas it is readily explained if the virus leaves the vascular system and is taken up from the tissues by nerve endings.

Finally Schweinburg (1932) a few hours after injecting the virus intravenously attempted to identify it in various organs. He found that whereas the spleen contained the virus in 9 of 25 experiments the

brain gave negative results throughout. At first sight this experiment appears conclusive. Schweinburg himself, however, pointed to the well known fact that even after intracerebral injection some viruses cannot be identified in the brain for many hours after their injection.

As may be seen from this summary, the question whether the virus of rabies reaches the central nervous system directly from the blood cannot yet be answered with certainty. There exists, however, some presumptive evidence that it does not.

Borna Disease.—Zwick, Seifried and Witte (1929) and Nicolau and Galloway found that Borna disease can be produced in rabbits by injecting the virus into the sciatic nerve or into the brachial plexus. After injection into the central nervous system the virus was found in the peripheral nerves (in larger quantities in the proximal than in the distal parts). The neural transport of the virus therefore seems definite. It is questionable whether after peripheral injection the virus can be identified in the blood. Ernst and Hahn obtained positive results, while the experiments of Zwick, Seifried and Witte (1926) and of Nicolau and Galloway gave negative results. The latter authors were unable to infect rabbits by the intravenous route. The former obtained positive results only when the injections were repeated four times. An important observation was reported by Zwick, Seifried and Witte (1929). They found that rabbits could not be infected by the intracutaneous route. The results were usually positive, however, if serum or saline solution was injected simultaneously into the brain. These findings seem to indicate that after intracutaneous injection small quantities of virus reach the blood stream but are unable to pass the normal capillaries of the central nervous system. If these capillaries are damaged, however, infection occurs. If one compares this result with the difficulty with which rabbits are infected by the intravenous injection of large quantities of virus, it becomes unlikely that undamaged capillaries of the central nervous system are permeable to the virus of Borna disease.

Herpes Simplex.—The careful histologic investigations of Goodpasture and Teague, Marinesco and Draganesco, and Levaditi and Haber have shown that after intracorneal inoculation the virus of herpes simplex reaches the brain by way of the sensory portion of the fifth cranial nerve. Goodpasture and Teague injected the virus into the masseter muscle, the vitreous humor, the trachea, the skin, striated muscles, the liver, the spleen, the adrenal glands, the peritoneum and the ovaries and found histologic lesions and inclusion bodies only in the central pathways of the nerves that innervated the injected areas. These experiments leave no doubt that after injection into any kind of tissue the virus reaches the central nervous system exclusively by neural pathways.

It is certain, however, that with this mode of inoculation the virus does not appear in the blood with any regularity. On the contrary,

the experiments in which the virus could be identified in the blood have been rather rare exceptions.

Whether the virus of herpes simplex reaches the central nervous system directly from the blood, therefore, can be decided only by means of intravenous injections. The reports in the literature concerning this method of inoculation are conflicting. Many investigators obtained entirely negative results. There is no doubt, however, that with highly virulent strains encephalitis or myelitis has been produced by intravenous injection, although even under these conditions infection did not occur in all of the animals. Levaditi therefore assumed that the positive results are explained by the well known fact that approximately 25 per cent of rabbits suffer from spontaneous encephalitis (Encephalitozoon cuniculi) which renders the cerebral capillaries permeable to the virus. Doerr and Hallauer, however, expressed the belief that even in normal animals the virus reaches the central nervous system directly from the blood. It seems to me that the following observations are difficult to reconcile with this belief.

After injection of the virus into the brain the period of incubation for encephalitis is from three to seven days. After intravascular injection, however, Doerr and Zdansky found an incubation period of sixteen to twenty days and Teissier, Gastinel and Reilly one of thirteen to thirty days. Moreover, Doerr and Hallauer found the period of incubation to be the same (five to eleven days) whether the virus was injected into the vascular system or into the pads of the hindlegs of guinea pigs. Since in the latter case the virus reaches the central nervous system exclusively by way of the peripheral nerves, one would expect the period of incubation to be shorter after intravenous injection if the virus reached the central nervous system directly from the blood.

Experiments by Magrassi with a peculiar strain of the virus of herpes simplex are of some significance. While encephalitis developed in the animals after intravenous injection of the ordinary strains, this strain produced only myelitis. Since it was encephalitogenic by the intracerebral route, the peculiar localization of the virus in the lumbar cord would be difficult to understand if it reached the central nervous system directly from the blood. The result would be more plausibly explained by assuming that various strains have predilections for different nerves. Doerr and Hallauer based their opinion that the virus reaches the central nervous system directly from the blood on an interesting experiment. Strain M, which after intravenous injection produced only myelitis, was injected into the distal stump of a transected carotid artery. Of 5 rabbits given this type of injection, 3 showed encephalitis whereas 2 showed symptoms of myelitis. The authors concluded that in the first 3 animals virus had reached the brain directly by way of the cerebral capillaries.

It seems to me, however, that this experiment when correctly interpreted is important evidence for the indirect transmission of the virus of herpes simplex from the blood to the central nervous system. Friedemann and Elkeles showed in experiments with vitally staining dyes that injection into the ramifications of the internal carotid artery through the distal stump of the artery is impossible unless the pressure of injection is exaggerated. The pressure in the circle of Willis is so high that even after very rapid injection none of the injected dye reaches the cerebral arteries. The injected fluid finds its way entirely into the external carotid artery. Since Doerr and Hallauer emphasized that they injected the suspension slowly, it is almost certain that in their experiments no virus reached the cerebral capillaries. The fact that encephalitis developed in 3 of the animals can be explained only by assuming that the virus left the capillary bed of the external carotid artery and reached the brain by way of the cranial nerves. The existing experimental evidence tends to show that the virus of herpes simplex is unable to reach the central nervous system directly from the blood.

Pseudorabies.—That the virus of pseudorabies reaches the central nervous system by way of the peripheral nerves has been shown by a variety of experiments. Hurst (1933, 1934), using rabbits, injected the virus simultaneously in the neighborhood of the ear, the flank and the foot. Itching, characteristic of this disease, began in the region of the ear, started considerably later in the flank and was not observed in the foot because of the early death of the animal. Apparently the onset of this symptom was determined by the length of the nerve pathway. After the virus had been injected into the flank, histologic lesions (inflammation and inclusion bodies) were found only in the corresponding nerve, spinal ganglions and posterior horn cells. The virus could be identified in this part of the cord while the remainder of the central nervous system was still virus free.

Sabin inoculated the virus intranasally and found histologic lesions confined to the sensory division of the fifth cranial nerve, the parasympathetic and the sympathetic nerves. No lesions were found in the olfactory nerve and its central connections. This specific distribution of the lesions over certain neurons would obviously be impossible if at the same time the virus reached the brain by the vascular route. This conclusion is important since at least the Aujentsky strain of the virus of pseudorabies invades the blood early in the disease and multiplies rapidly in it. The experiments with pseudorabies therefore show clearly that this virus is unable to reach the central nervous system directly from the blood. Experiments of Hurst (1936) on monkeys strengthen this conclusion. This species is readily infected by the intracerebral injection of the virus, while the intramuscular and the intravenous injection are entirely innocuous.

Virus B.—The investigations of Sabin have shown conclusively that virus B when injected into the tissues reaches the central nervous system solely via the peripheral nerves. In rabbits the hindlegs became paralyzed whether the virus was injected into the skin, the muscles of the hindlegs, the peritoneum or the testicles. After corneal inoculation encephalitis developed. After the virus had been injected into the skin of the abdomen, the pooled lumbar, dorsal and cervical cords contained five hundred times more virus than the frontal lobes of the brain.

No virus could be identified in the blood after peripheral injection. After intravenous injection all four extremities became paralyzed. The existing experimental evidence is not sufficient to enable one to decide whether or not virus B reaches the central nervous system directly from the circulation.

St. Louis Encephalitis.—Webster and Clow showed that mice could be infected with the virus of St. Louis encephalitis by the intranasal route. Twenty-four to forty-eight hours subsequent to the inoculation, virus was found either in the olfactory bulb alone or in this structure and the piriform lobe. These experiments showed conclusively that after intranasal inoculation the virus of St. Louis encephalitis reaches the brain exclusively through the olfactory nerve. Only occasionally could the virus be identified in the blood after intranasal inoculation, but its constant presence in the spleen indicated that it had entered the blood at some stage of the infection.

Even 10^6 cerebral minimal lethal doses given intraperitoneally failed to produce encephalitis, although ten minutes, twenty minutes and one and three hours after the injection the blood contained large amounts of the virus (Webster). When, however, the cerebral capillaries were damaged by the intracerebral injection of sterile solutions of starch, encephalitis developed after intraperitoneal injection of the virus. (King recently reported that he could not reproduce these findings.) The experiments of Webster and Clow are very complete in showing that the virus of St. Louis encephalitis is unable to reach the central nervous system from the blood.

Louping Ill.—In the case of the virus of louping ill, the question is complicated by the fact that the genesis of the disease apparently is not entirely identical in different animal species. Hardly any investigations pertinent to the problem have been carried out in sheep, the natural host. Galloway and Perdrau infected monkeys by the intranasal route. Since with the exception of 1 animal no virus was found in the blood or visceral organs, they assumed that infection had taken place by neural routes. No definite pathway, however, could be demonstrated by identifying the virus in various parts of the brain. Two monkeys were given large doses intravenously. One animal remained in perfect health although virus was present in the blood for at least six days. The other

monkey became sick and was killed after forty-eight hours. Virus was identified in the blood, the spleen, the lungs and the mesenteric glands but not in the brain. These experiments, although few, seem to indicate that the virus of louping ill is unable to reach the central nervous system directly from the blood.

Burnet and Lush reported important experiments on mice. These animals were readily infected by the intranasal route, and on the second, third and fourth days the virus was found only in the olfactory bulb. Since Fite and Webster reported that virus was present in the blood from the second to the sixth day after intranasal instillation, the results of Burnet and Lush suggest that the virus does not reach the brain directly from the blood. Furthermore, after the intraperitoneal injection of large doses 90 per cent of the animals came down with encephalitis, but three to four days after the injection the virus was found almost exclusively in the olfactory bulbs. By treating the nasal mucous membrane with zinc sulfate, tannic acid or alum the survival time was considerably prolonged. These experiments tend to show that after intraperitoneal injection the virus of louping ill is excreted on the nasal mucosa and carried to the brain by way of the olfactory nerve.

Neurotropic Strain of Yellow Fever.—Findlay and Clarke (1935) found that monkeys and mice are susceptible to intranasal inoculation of the neurotropic strain of the virus of yellow fever. In the first stage of infection the virus and histologic lesions were found only in the olfactory bulb and the forebrain. Treatment of the nasal mucous membrane with chemicals gave considerable protection in mice (Findlay and Mahaffy). Since in the monkey, according to Findlay and Clarke (1935), large amounts of virus are found in the blood after intranasal inoculation, the results of Findlay and his colleagues show that the neurotropic strain of the virus of yellow fever is unable to reach the central nervous system directly from the blood. This conclusion is strengthened by the fact that with rare exceptions adult mice (Theiler) and monkeys cannot be infected by the intraperitoneal or by the subcutaneous route. According to Sawyer and Lloyd, however, infection occurs if the cerebral capillaries are damaged by an intracerebral injection of a solution of starch. It is interesting to note that according to Theiler and Findlay and Clarke (1935) very young mice and hedgehogs can be infected by the subcutaneous and by the intraperitoneal route.

Vesicular Stomatitis.—Sabin and Olitsky (1937 a,b,c; 1938 d,e) have shown that the virus of vesicular stomatitis when injected into various tissues of 15 day old mice gains access to the central nervous system exclusively by neural routes. After intranasal inoculation it appeared first in the rhinencephalon; after intraocular injection, in the diencephalon and the mesencephalon, and after injection into the muscles of the hindlegs, in the lumbar cord. The pathways of the virus were

determined more precisely with histologic methods. After intranasal inoculation lesions were found in the olfactory tracts; after intraocular injection, in the optic tract. In both cases the lesions extended to the cortical areas of these nerves. One year old mice could not be infected by the intranasal, the intraocular or the intramuscular route although the lethal dose by the intracerebral route was the same for young and old animals.

The authors emphasized that no systemic infection occurs after intranasal or intraocular inoculation. In young mice injection of the virus into the vein of the tail produced paralysis of the hindlegs. Sabin and Olitsky concluded that in this case the virus reached the cord by neural pathways, probably after having escaped into the subcutaneous tissue. In their entirety the experiments of Sabin and Olitsky indicate that the virus of vesicular stomatitis is unable to pass the capillaries of the central nervous system.

Equine Encephalomyelitis.—The interpretation of the experiments with the virus of equine encephalomyelitis meets with particular difficulties since the results are apparently determined by the species and the age of the experimental animal, possibly by the type of virus (Eastern or Western strain) and by the route of injection. The findings in the mouse are relatively clear. After intranasal instillation histologic lesions were observed only in the olfactory tract, and after intraocular injection, exclusively in the optic tract (Sabin, 1938; Sabin and Olitsky, 1937, 1938). When Eastern virus was inoculated into the muscles of the hindlegs of mice 15 to 21 days old flaccid paralysis of these limbs developed in 5 per cent of the animals, indicating that the virus had reached the central nervous system by way of the local nerve. All other animals died of encephalitis. Most of the mice, however, escaped involvement of the central nervous system if the nasal mucosa was treated with chemicals prior to the intramuscular injection of the virus. In the few animals in which encephalitis developed despite this treatment lesions were found in the cord and the medulla oblongata (spread via nerves supporting muscle), the central pathways of the auditory and vestibular nerves and the medullary nucleus of the seventh nerve. The experiments of Sabin and Olitsky in mice therefore tend to show that after intranasal and intraocular injection and only in exceptional cases after intramuscular injection the virus of equine encephalomyelitis reaches the central nervous system exclusively by peripheral nerves from the inoculated site. Since after intramuscular injection the virus invades the blood stream in most of the animals and may multiply in it and circulate for a few (one to two) days, it must be concluded that in the mouse the virus of equine encephalomyelitis is unable to reach the central nervous system through the capillary endothelium. All these experiments were carried out in young mice. Old mice are resistant to the intramuscular injection of the virus although when inoculated by the intracerebral route young and

old mice are equally susceptible and although in both age groups the virus circulates in the blood in the same phase. It is obvious, therefore, that in old mice the virus does not reach the central nervous system directly from the blood.

More difficulties are encountered in the interpretation of the experiments on guinea pigs. Hurst and also Howitt found that these animals can readily be infected by the intranasal route with Western virus. After injection of the virus into the muscles of the hindleg, histologic lesions were found predominantly in the frontal lobe of the brain. However, encephalitis was not prevented by removal of the olfactory bulbs and the anterior parts of the olfactory tracts. Hurst therefore assumed that the operation as such damaged the blood-brain barrier and thus, despite interruption of the normal olfactory pathway, made the brain accessible to the virus. In view of the fact, however, that the same operation prevents poliomyelitis after intravenous injection of large doses of this virus, the explanation of Hurst can hardly be accepted. It appears as if the virus of equine encephalomyelitis does reach the central nervous system by way of the olfactory nerve but may also use other routes. As a matter of fact, Sabin and Olitsky (1938 a) and King (1938) found that after injection of mouse passage Eastern virus into the hindleg lesions were scattered all over the central nervous system especially the neopallial cortex, which is rarely involved in the mouse. Lesions were present near damaged blood vessels. The endothelium of the capillaries was swollen and proliferated and contained inclusion bodies. The histologic observations of Sabin and Olitsky (1938 a) as well as those of King indicate that the Eastern virus grows through the vessels and involves the nerve cells around them (compare also Hurst). Old guinea pigs given 10^7 or fewer mouse cerebral lethal doses are resistant (Sabin and Olitsky, 1938 b). In an exceptional guinea pig which became infected, the lesions were not scattered but corresponded to the innervation of the leg into which the virus was injected.

The rather complicated results may be briefly summarized as follows: In young and old mice, and in old guinea pigs under special conditions, the virus of equine encephalomyelitis does not reach the central nervous system directly from the blood. In young guinea pigs the virus grows through the blood vessels and reaches nerve tissues by this route. In this connection Dr. P. K. Olitsky, of the Rockefeller Institute for Medical Research, gave valuable advice and suggestions.

Canine Distemper.—Experiments of De Monbreun seem to indicate that the virus of distemper reaches the central nervous system in dogs in essentially the same way as does that of equine encephalomyelitis in young guinea pigs.

Fowl Plague.—The virus of fowl plague may be included in this discussion although, according to the nomenclature of Hurst,[3] it is pantropic rather than neurotropic in the strict sense of the word. Doerr and Seidenberg (1932-1933), however, have shown that in the chicken the brain at the time of death contains much more virus than can be accounted for by the blood content of the organ. In view of the fact that virulent strains usually kill chickens within thirty to forty-eight hours it is unlikely that the virus reaches the brain by neural pathways. Moreover, in the guinea pig and the mouse the virus was identified in the brain three hours after its intravenous injection although it had disappeared from the blood within one to two hours. Doerr therefore assumed that the virus of fowl plague reaches the central nervous system directly from the blood.

It is of great interest that the results differed with other strains of the virus and in other species. Working with the less virulent Egyptian strain, Lagrange found that the course of the disease was much more protracted. In the second stage the virus was found in the brain, but in the first stage the brain was virus free for several days, although large amounts were found in the blood. Lagrange concluded from these experiments that the cerebral capillaries are impermeable to the Egyptian strain of the virus of fowl plague. Doerr and Seidenberg (1931-1932) were unable to identify the virus in the brains of rabbits into which it was injected intravenously. Theiler,[4] Kleine and Kleine and Moellers found that adult geese could not be infected by the subcutaneous or the intramuscular route, although the virus was highly pathogenic if given intracerebrally. These findings are very interesting from the point of view of the neurotropism of viruses. In this context one may confine oneself to stating that certain strains of the virus of fowl plague in certain animal species apparently are able to pass the capillaries of the central nervous system.

Summary of Recorded Results.—The results of the experiments discussed in this review may be summarized as follows: The blood-brain barrier is impermeable to the majority of neurotropic viruses. The evidence to that effect is conclusive or at least highly suggestive for the viruses of poliomyelitis, rabies, Borna disease, herpes simplex, louping ill, pseudorabies, St. Louis encephalitis, vesicular stomatitis, the neurotropic strain of yellow fever and for equine encephalomyelitis in mice and old guinea pigs. Virus B. when injected subcutaneously also reaches the central nervous system exclusively by the neural route, but for the time being it is impossible to ascertain that it is unable to pass the blood-brain barrier.

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3. The reference is to Hurst (1936) under "General Literature" in the bibliography.

4. The reference is to Theiler under "Neurotropic Stain of Yellow Fever" in the bibliography.

In young guinea pigs the virus of equine encephalomyelitis passes directly through the walls of the cerebral capillaries. But this passage cannot be considered a physicochemical process. It is mediated by the multiplication of the virus within the endothelial cells. The same mechanism is probably operative in the case of the virus of canine distemper.

The only virus to which the blood-brain barrier is permeable in a physicochemical sense is the pantropic virus of fowl plague. This, however, holds true only for highly virulent strains in chickens, mice and guinea pigs.

ELECTROCHEMICAL ASPECTS OF THE PROBLEM

It has been shown in the preceding section that in a physicochemical sense the capillaries of the central nervous system are probably impermeable to all neurotropic viruses with the exception of the virus of fowl plague. In looking for an explanation of these findings, one must in the first place consider the factor of size. Neurotropic viruses are corpuscular elements, and it might appear plausible that this factor prevents them from passing the capillary membrane. Quantitative considerations, however, cast some doubt on the validity of this assumption. As has been shown elsewhere, the capillaries of the central nervous system are permeable to antibodies. The elementary bodies of some viruses (those of poliomyelitis, louping ill, foot and mouth disease), however, are so small that they hardly exceed in size large protein molecules. Moreover, it has been seen that the capillaries of the central nervous system are permeable to the virus of fowl plague, the elementary bodies of which are not even particularly small.

In my review on the blood-brain barrier I showed that the ability of aniline dyes, toxins, antibodies and drugs to pass the capillaries of the central nervous system is determined by their electrical charge. The question naturally presents itself whether the same rule holds true for neurotropic viruses. A discussion of this question is complicated by the rather conflicting evidence concerning the electrical charge of neurotropic viruses at the hydrogen ion concentration of the blood. Obviously, the results of cataphoretic experiments may be vitiated by technical errors which have not always been taken into consideration. The most important of these errors is the interference of electroendosmotic water currents which shift the isoelectric point to the alkaline side. In the following tables I have recorded, therefore, only results obtained with the technics of Todd and Michaelis in which this error is as far as possible eliminated. Concerning the neurotropic viruses listed in the accompanying table reliable data are available in the literature.

As may be seen from the table, the neurotropic viruses thus far investigated carry a negative charge at the hydrogen ion concentration of

the blood. This would be in keeping with their inability to pass the capillaries of the central nervous system. In view of the fact, however, that most biologic products are negatively charged it may be questioned whether this agreement between experimental facts and theoretic expectation is more than a coincidence. The only virus known to pass through the capillaries of the central nervous system is the pantropic strain of the virus of fowl plague studied by Doerr and Seidenberg. The electrical charge of this virus, therefore, is of the greatest theoretic interest. I have not been able to find cataphoretic experiments with this strain of virus reported in the literature. Dr. A. Todd, of the National Institute for Medical Research, London, however, informed me about his own unpublished experiments. In contradistinction to all other viruses, the virus of fowl plague went neither to the anode nor to the cathode. Obviously, this virus is isoelectric at the hydrogen ion concentration of the blood. Experiments of Doerr and Gold point in the same direction.

Cataphoretic Experiments with Neurotropic Viruses

Virus	p_H	Charge	Author
Louping ill	7.3	Negative	Lépine (1931)
Borna disease	6.6-7.4	Negative	Nicolau and Kopciowska (1931)
Yellow fever	5.2-7.0	Negative	Hindle and Findlay
Herpes simplex	5.3-7.8	Negative	Nicolau and Kopciowska (1930 a,b)
Rabies	5.8-7.4	Negative	Nicolau and Kopciowska (1930 c)
Poliomyelitis	6.9-8.0	Negative	Levaditi and Lépine
Equine encephalomyelitis (Eastern strain)	Above 4.1	Negative	Finkelstein, Marx, Bridgers and Beard
Vesicular stomatitis	6.6-8.4	Negative	Olitsky and Cox (personal communication)

They found that the virus of fowl plague is much more strongly absorbed by the electronegative charcoal than are other viruses and bacteriophages. Since Friedemann and Elkeles have shown that the isoelectric toxin of lamb dysentery passes through the cerebral capillaries, the findings of Todd tend to show that the ability of viruses to pass these capillaries may, indeed, be determined by their electric charge. It has been seen that, according to Lagrange, the Egyptian strain of the virus of fowl plague is unable to reach the central nervous system through the capillary walls. It is of great interest that another French author, Lépine (1930), reported a negative charge for the virus of fowl plague over a range of p_H 6.2 to 8.2.

The experimental data concerning the electrical charge of viruses are not yet numerous enough to arrive at final conclusions. As far as the evidence goes, it is certainly not at variance with the assumption that in conformity with the results obtained with aniline dyes, toxins, antibodies and drugs, the ability of viruses to pass the capillaries may be related to their electrical charge. At least this hypothesis lends new significance to cataphoretic experiments with viruses. It is to be hoped

that in the future a more extensive experimental material obtained with a uniform and impeccable technic will be available.

It seems that the electrochemical concept of capillary permeability gives the only satisfactory explanation of the experimental findings reviewed in this paper. Obviously, the propagation of neurotropic viruses in the paths of certain neurons becomes understandable only if one considers the blood-brain barrier as completely impermeable to these viruses. Even a few elementary bodies passing through the capillary walls would infect the central nervous system and mar the clear picture of axonal spread. The difficulty in explaining this complete impermeability of the blood-brain barrier to neurotropic viruses along mechanical lines has induced Doerr to discard the whole theory of the blood-brain barrier. Obviously, this difficulty does not exist any longer once observers become reconciled to the idea that the permeability of the capillaries of the central nervous system is regulated by electrical forces.

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Notes and News

Appointments.—Herbert S. Breyfogle, a fellow in legal medicine at Harvard Medical School, has been appointed instructor in pathology at the Washington University School of Medicine and pathologist to the St. Louis County Hospital, effective May 1. Dr. Breyfogle will serve as pathologist to the coroner of St. Louis County.

Granville A. Bennett, of the department of pathology at Harvard Medical School, has been appointed professor of pathology and bacteriology at the school of medicine of the Tulane University of Louisiana, New Orleans.

Awards.—Vincent du Vigneaud, professor of biochemistry at the Cornell University Medical College, has been given the Mead Johnson & Company award of $1,000 for research on the vitamins of the B complex, in recognition of his work on biotin.

The Civic Medal awarded annually by the Rochester Museum Association was presented on May 13 to George H. Whipple, dean of the University of Rochester School of Medicine and Dentistry.

The Theobald Smith Award of $1,000 and a bronze medal of the American Association for the Advancement of Science, established by Eli Lilly & Company in 1935, has been given to Sidney C. Madden, assistant professor of pathology at the school of medicine and dentistry of the University of Rochester, in recognition of his work on plasma proteins.

National Academy of Sciences.—Warfield T. Longcope, of the department of medicine at Johns Hopkins University, and O. H. Robertson, of the department of medicine at the University of Chicago, have been elected members of the Academy.

Deaths.—Harry Gideon Wells died April 26 at the age of 67 years. James Ewing died May 16 at the age of 76 years. Obituary sketches of Dr. Wells and Dr. Ewing will appear in a forthcoming number.

Hormones.—A symposium on hormones will be held at Gibson Island July 19 to July 23 in connection with this summer's conferences under the auspices of the American Association for the Advancement of Science.

Foundation for Infantile Paralysis.—This foundation has made long term grants for the study of infantile paralysis and related diseases as follows: John Hopkins University, $300,000; Yale University, $150,000; University of Michigan, $230,000. A bibliography of the scientific literature on infantile paralysis is in course of preparation.

Books Received

TWENTY YEARS OF MEDICAL RESEARCH. Dorothy White Nicolson. Pp. 97. New York: Medical Research Committee, National Tuberculosis Association, 1943.

This is an instructive and comprehensive review of the work of the Committee on Medical Research (William Charles White, chairman) of the National Tuberculosis Association. The director of the association, Kendall Emerson, states in the preface that the outcome of the work here reviewed "proves the effectiveness of the policy adopted from the beginning, a logical selection of topics for study, seeking the expert best qualified to carry forward a special phase of the research, enlisting the interest of universities in providing necessary facilities." The survey and the bibliography of the researches aided by the committee, which clearly justify Dr. Emerson's statement, will be of interest to those who are concerned with cooperative research in general and with research in tuberculosis in particular.

PROTEIN HORMONES OF THE PITUITARY BODY. H. B. Van Dyke, Bacon F. Chow, Vincent du Vigneaud, H. L. Fevold, George W. Irving Jr., C. N. H. Long, Theodore Shedlovsky and Abraham White. Annals of the New York Academy of Sciences. Volume 43. Pp. 253-426. New York: New York Academy of Sciences, 1943.

This volume consists of a valuable series of papers presented at a conference on the protein hormones of the pituitary gland in the section of physics and chemistry of the New York Academy of Sciences. The authors and topics of the papers follow: H. B. Van Dyke, introduction; Theodore Shedlovsky, criteria of purity of proteins; George W. Irving Jr. and Vincent du Vigneaud, hormones of the posterior lobe of the pituitary gland; Bacon F. Chow, the chemistry of "thylakentrin," the follicle-stimulating hormone of the anterior lobe of the pituitary gland; H. L. Fevold, the luteinizing hormone of the anterior lobe of the pituitary gland; Abraham White, the lactogenic hormone and mammogen; C. N. H. Long, the growth-promoting and metabolic hormones of the anterior lobe of the pituitary gland.

CLINICAL SIGNIFICANCE OF THE BLOOD IN TUBERCULOSIS. Gulli Lindh Muller, M.D., pathologist and director of laboratory, New England Hospital for Women and Children, Boston; formerly pathologist, Rutland State Sanatorium, Rutland, Mass. Pp. 516. Price $3.50. New York: The Commonwealth Fund, 1943.

The basis of this book is complete hematologic studies of 1,000 cases of tuberculosis (6,819 complete examinations) coupled with a correlation of the literature on the blood in tuberculosis. The book is divided into five parts: the physiology of the blood-forming organs and the cellular response to the tubercle bacillus; changes in the circulating blood in tuberculosis; the sedimentation rate; clinical and hematologic data as measures of the constitutional reaction; the effect of therapeutic measures, exercise and certain complications on the hematologic picture; methods for the examination of the blood. There are numerous tables and charts. The book is an important addition to the literature in its field.

OUTLINE OF ROENTGEN DIAGNOSIS: AN ORIENTATION IN THE BASIC PRINCIPLES OF DIAGNOSIS BY THE ROENTGEN METHOD. Leo G. Rigler, B.S., M.B., M.D., professor of Radiology, University of Minnesota, Minneapolis. Pp. 196, with 227 figures, presented in drawings and reproductions of roentgenograms. Figures 6 to 51 and 55 to 72 are drawings in an original technic by Jean E. Hirsch. Philadelphia: J. B. Lippincott Company, 1943.

The text has been revised and expanded to include recent advances in the technic, the methods and the scope of roentgen examination, but the size of the book has not been increased. The book will maintain its standing as an excellent outline for the teaching of roentgen diagnosis.

THE SIGHT SAVER. C. J. Gerling. Pp. 202. Price $2. New York: Harvest House, 1943

This book, organized like a dictionary or an encyclopedia, deals comprehensively and accurately with the eye and with conservation of sight. In no sense does it tend to replace the physician or the ophthalmologist but presents tellingly the dangers to vision of fraud and quackery. The language is clear and simple. The book should be widely used.

A Manual of Pulmonary Tuberculosis (Part I) and An Atlas of Thoracic Roentgenology (Part II). David O. N. Lindberg, M.D., F.A.C.P., lecturer on tuberculosis, State University of Iowa College of Medicine; director of roentgenology, State Sanatorium, Iowa Pp. XVI and 219 plus an index, with 189 illustrations, including 145 plates. Price $6.50. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

Dr. Lindberg has produced a concise, well illustrated description of the diagnosis, the general and the surgical treatment, and the control of pulmonary tuberculosis, supplemented by an atlas (145 figures) of thoracic roentgenology.

La Urobilina en el estado normal y patológico. Marcelo Royer. Trabajo del Instituto de fisiología Facultad de ciencias médicas, Buenos Aires. Second edition. Pp. 265, with 43 figures. Buenos Aires: Editor "El Ateneo," 1943.

The first edition was published in 1929, and a French edition appeared in 1930. A full account is given of human urobilin under physiologic and pathologic conditions, its determination, its variations, its elimination and its significance in tests of hepatic function.

Chemotherapy of Gonococcic Infections. Russell D. Herrold, B.S., M.D., associate professor of surgery (urology), University of Illinois College of Medicine, Chicago. Pp. 137. Price $1. St. Louis: C. V. Mosby Company, 1943.

An important, timely, practical contribution to the effective treatment of gonococcal infections, based on the personal observations of the author.

Dictionary of Bio-Chemistry and Related Subjects. Edior in Chief, William Marias Malisoff, professor of biochemistry at the Polytechnic Institute of Brooklyn. Pp. 579. Price $7.50. New York: Philosophical Library, 1943.

A Guide to the Preservation of Life at Sea After Shipwreck. Medical Research Counccil, Committee on the Care of Shipwrecked Personnel: War Memorandum No. 8. Pp. 21. Price 10 cents. London: His Majesty's Stationery Office, 1943. (The British Library of Information, 30 Rockefeller Plaza, New York.)

School of Tropical Medicine Under the Auspices of Columbia University, San Juan, Puerto Rico. Report of the Director for the Year Ending June 1942. Published by the University of Puerto Rico and Columbia University.

The National Foundation for Infantile Paralysis, Inc. Annual Report, 1942. Pp. 55. New York: 120 Broadway.

Ten Years' Progress in Cancer Research. A Symposium Commemorating the Tenth Anniversary of the International Cancer Research Foundation. Pp. 53. Philadelphia: The International Cancer Research Foundation.

Hope Deferred. Jeanette Seletz. Pp. 536. New York: The Macmillan Company, 1943.

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